HENRY FORD HOSPITAL

International Symposium

Biology of Pyelonephritis

The symposium was sponsored by the Henry Ford Hospital, Detroit, Michigan, and held at the hospital October 8, 9, 10, 1959.

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HENRY FORD HOSPITAL INTERNATIONAL SYMPOSIUM

Biology of Pyelonephritis

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Foreword

The decision that pyelonephritis was a proper subject for an International Symposium was based on the renewed interest in this disease, as evidenced by the increasing number of contributions from many investigators, representing different disciplines.

With the approval of the staff of the Henry Ford Hospital, the Local Program Committee was formed, and enlisted the aid of the members of *the Advisory Committee, consisting of recognized authorities in this field.* The results of the deliberations of this committee are set forth in the Contents of this publication

An early decision of the Program Committee was to explore certain areas of inquiry in a relatively systematic manner. The major areas selected included origin, development, structure, function, and distribution – and these by definition characterize the science of biology Thus the title of this Symposium was formulated.

Next, the consideration of specific participants was undertaken with the full realization that it would be impossible to include many who had made significant contributions to the study of pyelonephritis We trust, however, that those essayists who agreed to present their material at this forum have provided food for thought, and have suggested new lines of investigation, or possibly given a new understanding to the interrelationships of the various aspects of the biology of pyelonephritis

Finally, the provision of adequate time for general discussion was agreed to be of major importance and the program was so oriented

It is therefore the hope of the Henry Ford Hospital staff and of the Program and Advisory Committees that those individuals who attended the three-day session and those who read the published volume of the proceedings will find something that is informative, stimulating and of scientific and personal value

EDWARD L. QUINN, M D.
EDWARD H. KASS, M D

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I

Pathogenesis of Pyelonephritis

Chairman PAUL B. BEESON, M.D. (*New Haven, Connecticut*)

the total number of bacteria trapped in an obstructed kidney is measured by the count per gram times the total weight. If this precaution was taken, the obstructed kidney was found to contain about twice as many bacteria as the normal, and this was seen both in the cortex and the medulla. Following the fate of this implant it was observed that in the medulla the bacteria increased in number without delay and remained in the logarithmic phase of growth for the five hours studied. However, the mean generation time (40 minutes) was about twice that obtained in broth. In the case of the cortex there was a lag of some 90 minutes followed by a similar logarithmic phase of growth When the growth curves for cortex and medulla were superimposed, it was found that the number of bacteria in the cortex began to increase when the number in the medulla equaled that in the cortex. The two zones then proceeded in step and it was possible that the cortex was in fact being filled from below

Chronic Obstruction

If the ureter had been obstructed for one to six days before the bacteria were injected, the changes in bacterial count were somewhat different. The medulla appeared to behave as in the acutely obstructed kidney, but the numbers in the cortex showed relatively little increase over the five hours. If, as suggested, the cortex is filled from the medulla, it would seem that the tubules are no longer open to retrograde spread of infection.

Effect of Removal of Obstruction

The results may be summarized by saying that in the seven days following removal of the ligature the kidneys changed from an obstructive pattern to the normal control pattern. This change was accompanied by a loss of the retained fluid and there was good correlation between susceptibility to infection and increase in weight

To summarize the evidence presented so far, one might say that obstruction will render a kidney susceptible to bacterial infection in a matter of minutes The environment of the kidney changes to such a degree that bacteria quiescent in the normal are able to multiply in the logarithmic phase in the obstructed While it is true that the mean generation time of the bacteria is prolonged, this might be due to escape or destruction of bacteria rather than depression of metabolic activity. It would seem that this change correlates well with the increase in weight due to retained fluid, and it suggests that there is need for research into the method by which this retained fluid acts Some suggested modes of action are (*a*) accumulation of suitable nutrients, (*b*) prevention of white cell immigration, (*c*) depression of the kidneys' normal bactericidal and/or bacteriostatic mechanisms

There are, however, one or two points that merit discussion. Firstly, as seen above, chronic obstruction appears to protect the cortex from infection as compared with the acute state. Secondly, chronic partial obstruction of the ureter in rats and rabbits [11] was not found to render the kidney highly susceptible to infection. On the other hand, the condition of intrarenal hydronephrosis due to scarring was found to predispose to infection of the obstructed segment for months.[3] This has been confirmed by Rocha *et al.*,[10] who showed, using a cautery, that obstruction produced in the medulla predisposed the affected segment of the kidney to infection, while a lesion in the cortex did not.

There is therefore the possibility that chronic partial obstruction of the whole kidney may have a different result from chronic intrarenal hydronephrosis. While it is generally assumed that obstructed kidneys are highly susceptible to bacterial infection, most of the evidence is based on results obtained with acute obstruction. This is obviously an important clinical point which needs to be investigated.

Acute and Chronic Staphylococcal Infection of the Kidney

Acute Experiments

Coagulase-positive staphylococci are able to infect the kidney following intravenous injection. This is seen in rabbits[2] and in mice.[6] In man staphylococcal infection of the kidney is not uncommon, and in these three examples it appears that it is the normal intact kidney which is being attacked. Hence a study of the interaction between staphylococci and the kidney would be of interest both as a model of a human disease and as a study of the interaction of the kidney and a natural pathogen

The findings in a study of staphylococcal infection of the mouse kidney [9] were as follows·

(1) There is a roughly linear relationship between the logarithm of the dose injected intravenously and the number of mice dying or the number of kidneys infected.

(2) There is a linear relationship between the logarithm of the dose injected and the implant in a kidney.

(3) Hence there is a linear relationship between the logarithm of the number of staphylococci implanted in the kidney and the likelihood of infection occurring.

(4) With any size of implant there are always many fewer abscesses than the number of bacteria deposited. With suitable doses some kidneys escape entirely.

(5) It can be shown that the distribution of infected kidneys in groups of mice is satisfactorily predicted by the binominal formula $(p + q)^n$ where p = the proportion of kidneys infected, q = noninfected, and n = the number of kidneys in the animal, 2 For example, where $p = 0.5$ (that is, 50 per cent of the kidneys are infected) the distribution is 25:50:25 – that is, 25 per cent of mice have both kidneys infected, 50 per cent have one kidney infected, and 25 per cent escape infection.

(6) The need for an implant larger than the number of abscesses produced was studied. Two possibilities were explored:

(*a*) That the implant was not homogenous but contained a few virulent cells. It proved impossible by passage experiments to isolate such hypothetical mutants.

(*b*) That abscesses follow only the chance aggregation of several staphylococci so close together that they are able to offer one another mutual support. This theory was tested by injecting mice with mixtures of two or three different strains of staphylococci. It would be expected that if more than one staphylococcus was necessary for abscess formation, a considerable number of abscesses would be derived from cells of two or three strains. However, over 90 per cent of abscesses when tested yielded pure cultures. Hence it is unlikely that very much mutual support is involved in establishing an abscess.

Long-term Experiments

Use was made of the binomial formula to set up large groups of mice with a known degree of kidney infection in order to study the evolution of the staphylococcal lesion for many months. A dose was chosen following which about 40 per cent of the mice died of acute infection Of the 60 per cent remaining, one-third had bilateral disease, one-third unilateral, and the remainder escaped visible infection[10]

In the [illegible] only a few staphylococci. However, staphylococcal carriage in the kidneys persisted in a few mice and both coagulase-positive and coagulase-negative strains have been isolated as late as 420 days after infection. In a few mice death was due to acute staphylococcal infection developing as late as 300 days in a solitary cystic kidney. The origin of these organisms was not clear, but they may well have been persisters which for some reason became active. The problem of the origin of these persistent staphylococci is being studied at the moment by trying to identify them by their antibiotic and phage resistance patterns to see if they are identical with the original strain. At the same time studies of the virulence of these organisms are being made.

Histologic Changes

The earliest lesions were acute abscesses in the cortex, some of which spread down into the medulla in the first few days. As the acute lesion developed, areas of necrosis appeared outside the zone of suppuration. Sometimes coalescence of adjacent areas led to massive necrosis of the renal papilla. The period of acute infection was followed by healing, and histologic evidence of the persistence of the original bacterial infection was rare. Healing was accompanied by scarring and the main changes noted were (*a*) atrophy with or without replacement fibrosis, (*b*) hydronephrosis, (*c*) cystic changes.

(*a*) Atrophy. In extreme cases the kidney disappeared. This condition of "bacteriological nephrectomy" was found in about 10 per cent of mice infected over 150 days It is probably due to destruction of the renal papilla with resulting loss of function of the whole organ. In less severe cases the kidney was represented by a small nodule which contained granulation tissue and a few atrophied glomeruli. More commonly the atrophy was focal in one or both kidneys.

(*b*) Hydronephrosis. Dilatation of the nephron was seen as early as the twelfth day, while dilatation of the pelvis or ureter was not seen before the fiftieth day and it became greater with time.

(*c*) Cystic changes. These were characteristic of the late stage and consisted of progressive dilatation of the capsular space with disappearance of the glomerular tuft. These cysts varied in size from slightly larger than a glomerulus to as large as half the kidney.

Other Factors

In addition to the factors influencing the establishment of staphylococcal infection of the kidney outlined above, a number of other observations have been made. The effect of diet has been studied by Dubos and his colleagues. Smith and Dubos[17] showed that starvation for 36 to 48 hours increased susceptibility to infection. Later experiments[4] showed that mice kept on diets deficient in certain amino acids had diminished resistance and this effect could be reversed by a suitable amino acid supplement Other studies[18] showed that mice given dinitrophenol or thyroid extract were rendered more susceptible to infection. It is apparent from these observations that the establishment of staphylococcal pyelonephritis may be influenced by the occurrence of other diseases or by malnutrition.

Another problem which is suggested by the staphylococcal studies is that of the prolonged renal carriage of bacteria. This is not restricted to staphylococci and has been reported with a number of other organisms, for example *Salmonella typhimurium*.[13] If, as seems possible in mice, recrudescence of infection may be caused by activation of carried bacteria,

it would be important to study both the mechanism by which the bacteria persist and whether attenuation of virulence is produced If fully virulent bacteria persist for months, methods of sterilizing the kidney will have to be devised.

Summary

The limitations of present experimental models used to study pyelonephritis were considered. Two models, *Escherichia coli* and the obstructed rabbit kidney, and *Staphylococcus aureus* and the intact mouse kidney, were studied for the information they provided about the establishment of infection Some of the problems which remain in this restricted field were discussed.

In addition, the staphylococcal model was used to study the changes that follow healing of acute infection A wide range of histologic changes were seen The possibility that chronic staphylococcal carriage may occur and give rise to reinfection is described

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2

*Experimental Pyelonephritis: Observations on the Course of Enterococcal Infection in the Kidney of the Rat**

LUCIEN B. GUZE, M.D

(Los Angeles, California)

Studies of the pathogenesis of experimental pyelonephritis have been limited because of lack of a suitable model. Although such factors as ureteral obstruction, mechanical trauma, localized injury to the medulla, antecedent staphylococcal infection with resultant scarring, and desoxycorticosterone-induced arteriolar sclerosis will predispose the kidneys of experimental animals to pyelonephritis, the investigator is frequently unable to distinguish between the effects of the predisposing injury and the induced infection. The few microorganisms capable of initiating pyelonephritis in the "nonmanipulated" kidney usually cause rapid death of the experimental animal, or the renal lesion becomes bacteriologically sterile and the disease does not progress. Following the intravenous injection of a strain of enterococcus (*Streptococcus faecalis*) into normal rats, acute pyogenic infection of the kidney occurred. During the past two and one-half years, we have studied the host-parasite relationship of this infection. The present data describe the course and natural history of this experimental pyelonephritis in the rat.

White male Wistar strain rats weighing 100 to 200 Gm. were used throughout these experiments. The culture employed was a strain of *Streptococcus faecalis* originally isolated from the urine of a patient with pyelonephritis. Before using it in the experiments to be described, the organism was "passed" through a series of animals by injecting the culture intravenously into normal rats. One week after inoculation the kidneys were removed, homogenized and cultured, and the bacteria obtained were then injected into another animal. After three such animal passages, the organism was grown in beef heart infusion broth for 6 hours, 5 ml portions

*From Wadsworth Hospital, Veterans Administration Center, Los Angeles, and the Department of Medicine, University of California at Los Angeles Medical Center, Los Angeles, California. This work was supported by research grants from the National Institutes of Health, United States Public Health Service (E-1257), and the Southern California Chapter of the National Kidney Disease Foundation.

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White male Wistar strain rats weighing 100 to 200 Gm were used throughout these experiments. The culture employed was a strain of *Streptococcus faecalis* originally isolated from the urine of a patient with pyelonephritis. Before using it in the experiments to be described, the organism was "passed" through a series of animals by injecting the culture intravenously into normal rats. One week after inoculation the kidneys were removed, homogenized and cultured, and the bacteria obtained were then injected into another animal. After three such animal passages, the organism was grown in beef heart infusion broth for 6 hours, 5 ml. portions

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then being frozen and stored at −20° C. Each week one tube was warmed to 37° C., incubated for 24 hours, then "passed" once more through a rat. The organisms isolated, after proper identification, were used for all experiments during the next week. The tail vein was employed for all intravenous injections. The volume of culture injected was 1 ml. of an 18- to 24-hour culture. At the time of animal sacrifice, the whole kidney or whole spleen was cultured, in the case of the liver, approximately 1 Gm. of tissue was removed. The tissues obtained were homogenized and cultured quantitatively.

Course of Enterococcal Infection in Normal Animals

At intervals of 1 hour to 57 weeks after the intravenous injection of 400 to 450 million microorganisms, groups of 4 to 12 rats were sacrificed and tissues removed for study. Results of bacteriologic examination of the kidneys, spleen, and liver are presented in Figure 1. It should be men-

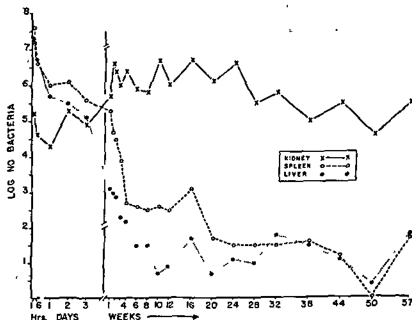


FIGURE 1. Bacterial population in the various organs of the normal rat following the intravenous injection of enterococci The points plotted represent the averages of the logarithms of the numbers of organisms cultured from the whole kidney or spleen, or from 1 Gm. of liver.

tioned that this inoculum was well tolerated. None of the animals died in the immediate postinjection period and the rats continued to eat and gain weight in a normal fashion.

The course of the infectious process was characteristic for each tissue, and there was little individual variation from animal to animal. The findings indicate that while large proportions of the intravenous inoculum were arrested in the liver and spleen, the kidneys participated only to a small degree in the removal of bacteria from the circulating blood. In the liver and spleen, destruction of the microbial population occurred at a relatively constant rate during the first 4 to 6 weeks. Thereafter, reduction in number of bacteria was more gradual. The first instances of negative cultures were noted at 6 weeks in the liver and at 20 weeks in the spleen. At subsequent times, failure to isolate bacteria from the liver and spleen occurred in occasional animals. In contrast, there was little evidence of killing of bacteria by the kidney. The microorganisms in this organ multiplied, attained a maximum population at 10 days, and thereafter persisted in relatively constant numbers throughout the period of observation. In 11 rats examined between 1 and 24 weeks after injection, one of the kidneys cultured revealed no growth at the lowest dilution tested. This usually represents less than 100 or 1000 bacteria per tissue studied. At 28 weeks, one animal was examined in which cultures of one kidney were negative (less than 10 bacteria per kidney) despite the presence of macroscopic and microscopic evidence of chronic inflammation and scarring. The other kidney presented bacteriologic and pathologic findings of infection. This observation of unilateral "burning out" of renal infection was noted in 4 of 27 animals examined between 32 and 57 weeks.

At the time of sacrifice, urine was obtained by direct needle aspiration of the bladder. Table 1 presents the results of bacteriologic examination of the urine during the first 5 days after inoculation. In 9 animals examined and 2 days after the intravenous injection of bacteria, urine cultures were egative. This occurred at a time when the microbial population of the dney was high. The first appearance of bacteriuria was noted on the rd day, and this persisted in animals examined on the fifth day. (The gative urine culture noted on the fifth day occurred in an animal in ch there was no bacteriologic evidence of renal infection. This sugted a faulty intravenous injection.) These data indicate that the kidney s not act as a "filter" for bacteria, rather, that infection with associated e destruction and rupture of microabscesses into the tubular collect- stem is necessary for the occurrence of bacteriuria. Subsequent urine res obtained at intervals up to 57 weeks after infection revealed a tently high level of bacteriuria. In the great majority of animals, than 100,000 viable enterococci per milliliter of urine were found. instance was a negative urine culture obtained in an animal which dence of pyelonephritis.

t blood cultures were obtained in 15 rats 1 to 7 days after inus inoculation, and in all of these enterococci were found. None

TABLE I. OCCURRENCE OF BACTERIURIA FOLLOWING THE INTRAVENOUS INJECTION OF ENTEROCOCCI INTO THE RAT

Time After Injection (days)	Logarithmic Numbers of Enterococcus Colonies Recovered	
	Kidney	Urine (ml)
1	3.6	0*
	3.6	0
	4.6	0
	4.6	0
2	3.6	0
	5.6	0
	3.3	0
	5.9	0
	3.5	0
3	6.5	5.4
	6.6	4.8
	5.0	4.4
	6.7	5.0
5	6.5	4.2
	5.9	6.9
	7.4	4.8
	0	0

* In these experiments, 0 means that no organisms were cultured from 1 ml of urine

f the 4 animals examined at 10 days had evidence of continuing bac-eremia. Blood cultures were negative in the 23 random examinations per-ormed between 3 and 57 weeks after infection. Thus, despite the evidence f persistent and continuing infection in the kidneys of infected rats, there as no evidence of continuing dissemination of bacteria into the general rculation.

omparative Numbers of Organisms in the Normal Renal Cortex nd Medulla at Different Intervals After Intravenous Injection Enterococci

To determine the distribution and fate of bacteria in the different parts the kidney during the course of infection, pieces of cortex and medulla ere removed separately and weighed on a Mettler analytical balance, pe B6, at varying intervals after the intravenous injection of bacteria. ne weights of the tissues cultured ranged between 90 and 145 mg. for

the cortex and between 8 and 19 mg. for the medulla. The results of these bacteriologic studies are presented in Figure 1.

Although a large number of enterococci initially localized in the renal cortex, this part of the kidney appeared able to temporarily "handle" this inoculum, as manifested by a decrease in the bacterial content during the first 2 days. The resistance of the medulla, however, was not as ef-

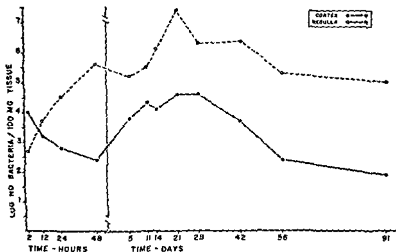


FIGURE 1. Bacterial population in the cortex and medulla of the normal rat kidney following the intravenous injection of enterococci. The points plotted represent the averages of the logarithms of the numbers of organisms cultured.

ficient. Microbial multiplication occurred immediately in this area, and a relatively high population was attained at 2 days. After this time, the medullary bacterial count remained greater than that of the cortex throughout the period of observation. It should be mentioned that the earliest histologic lesions were observed in the medulla 2 to 3 days after intravenous injection. This coincided with the period of rapid bacterial multiplication in this area. Abscesses appeared in the cortex between the fourth and fifth days, at a time when there was a secondary rise in the number of enterococci in this part of the kidney. It is not apparent from these data whether this increase noted in the cortex represents a proliferation of microorganisms originally lodged in this area or indicates a spread of infection from medulla to cortex.

Determination of the Number of Organisms Required to Cause Infection in the Normal Kidney

To determine the infecting dose necessary to produce infection in the normal rat kidney, groups of animals were given 1 ml. injections of serial tenfold dilutions of enterococcus culture, and 1 week later the kidneys were homogenized and cultured quantitatively. The results are presented in Table II An intravenous inoculum of 400 to 450 million organisms

TABLE II. Relation of Number of Enterococci Injected Intravenously to Incidence of Pyelonephritis in the Rat

Dilution (and Approximate Number of Organisms Injected)	Number of Animals	Number in Which Pyelonephritis Occurred
Undiluted (400–450 million)	14	14
10^{-1} dilution (40–45 million)	8	4
10^{-2} dilution (4–4 5 million)	8	1
10^{-3} dilution (400–450 thousand)	12	1
10^{-4} dilution (40–45 thousand)	8	1

caused infection in all 14 of the animals studied. In other experiments not recorded here, injection of this number of bacteria was uniformly successful in introducing infection in more than 200 animals tested. When the number of bacteria injected was reduced, the proportion of animals infected diminished. At dilutions of 10^{-2} or less, only an occasional rat developed pyelonephritis

The results obtained in these experiments are of interest when examined with the data shown in Figure 1 An intravenous inoculum of 400 to 450 million organisms resulted in a bacterial population of approximately 100,000 in the kidney at 1 hour. If the proportion of injected bacteria lodging in the kidney does not change when the inoculum is reduced, then an inoculum of 10^{-3} or less would result in fewer than 100 microorganisms being deposited in the kidneys. This type of data permits the establishment of an estimated tissue population necessary to produce infection. Thus, in animals receiving 40 to 45 million organisms (1 ml of 10^{-1} dilution), the infecting tissue population which caused pyelonephritis in 50 per cent of animals is calculated to be approximately 10,000 viable units.

Ability of Various Group D Streptococci to Produce Pyelonephritis in the Rat

Observations were made on the occurrence of renal infection following the inoculation of various group D streptococci into normal rats. The

results of these experiments are presented in Table III. Three strains of *Str faecalis*, two of which were recently isolated from the urine of patients with pyelonephritis, produced infection in the majority of animals tested. Similar results were obtained with strains of *Str. faecalis* var.

TABLE III. INCIDENCE OF PYELONEPHRITIS FOLLOWING THE INTRAVENOUS INJECTION OF GROUP D STREPTOCOCCI INTO THE RAT

Species	Source	Number of Animals	Number Infected
Str faecalis	ATCC 10541	10	9
Str faecalis	pyelo urine	9	9
Str faecalis	pyelo urine	10	9
Str faecalis var *zymogenes*	ATCC 6055	10	8
Str faecalis var *liquefaciens*	ATCC 13398	5	5
Str durans	ATCC 6056	10	0

zymogenes and *Str. faecalis* var *liquefaciens*. The single culture of *Streptococcus durans* tested failed to produce renal infection in any of the animals injected The inability of this strain of *Str. durans* to produce pyelonephritis may offer a tool with which individual parasite factors affecting the renal host resistance may be studied. This organism differs from *S.r. faecalis* in its failure to decolorize litmus milk, its lower saccharolytic activity, its sensitivity to tellurite, and its lower tyrosine decarboxylase activity. The role these and other differences might play in the pathogenesis of infection is at present under investigation.

Effects of Ureteral Obstruction on the Bacterial Population of the Kidney

Since obstruction to the flow of urine has been shown to predispose the kidney to pyelonephritis, an attempt was made to determine the effects of this manipulation on the course of enterococcal infection in the rat An 18- to 24-hour culture of *Str faecalis* was injected intravenously into rats 24 hours after ureteral ligation At varying intervals thereafter, groups of animals were sacrificed and their kidneys examined bacteriologically. The results are presented in Figure 3 At 1 hour after injection, the number of bacteria found in the obstructed kidney was similar to that of the normal organ. This would indicate that no increased "trapping" of bacteria from the circulating blood occurred in the kidney as a consequence of the ureteral obstruction Thereafter, the microbial population in the obstructed organ increased and reached a peak at 9 days after injection Subsequently, the number of organisms isolated from the hydronephrotic kidney decreased. By the thirtieth day, several of the kidneys examined had been converted to an enlarged sac of pus with little remaining renal parenchyma In this environment it is not surprising that the microbial

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The results obtained in these experiments are of interest when examined with the data shown in Figure 1. An intravenous inoculum of 400 to 450 million organisms resulted in a bacterial population of approximately 100,000 in the kidney at 1 hour. If the proportion of injected bacteria lodging in the kidney does not change when the inoculum is reduced, then an inoculum of 10^{-3} or less would result in fewer than 100 microorganisms being deposited in the kidneys. This type of data permits the establishment of an estimated tissue population necessary to produce infection. Thus, in animals receiving 40 to 45 million organisms (1 ml of 10^{-1} dilution), the infecting tissue population which caused pyelonephritis in 50 per cent of animals is calculated to be approximately 10,000 viable units.

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renal medulla and papilla. These increased in size during the next 2 to 3 days and in some instances ruptured into the tubular collecting system. During the first 3 to 4 days after infection, no lesions were noted in the cortex. Thereafter, abscesses were seen in the cortical interstitial tissue. Throughout this period, the glomeruli remained remarkably clear of evidence of infection. After the fourteenth day, the abscesses began to resolve, as manifested by lymphocytic infiltration, diminution in the number of polymorphonuclear leukocytes, and the appearance of macrophages Fibroblasts were seen between the second and fourth weeks, and thereafter healing and scar formation were noted As this process progressed, areas of dilated tubules with flattened lining epithelial cells, occasionally filled with pink-staining amorphous material, were observed between and adjacent to the scars. In the animals studied between the fiftieth and fifty-seventh weeks, an occasional instance of beginning periglomerular fibrosis was seen In all of the renal sections examined histologically, the blood vessels appeared normal.

Despite the occurrence of marked changes in the kidney and persistent bacteriuria, the alterations in the urinary bladder were minimal In occasional animals, submucosal focal collections of lymphocytes were noted In no instance was evidence of acute cystitis or disruption of the epithelial lining observed

Blood Pressure Changes in Chronic Pyelonephritis

Because of the reported association of pyelonephritis and hypertension, experiments were designed to study this relationship. Ninety rats were infected by the intravenous injection of enterococci and left undisturbed for 4 months. Thirty uninfected control animals were similarly handled At the end of this period, the infected animals were divided into the following groups

Group D received repeated intravenous injections of the enterococcus culture every 2 months

Group A received repeated intravenous injections of an *Escherichia coli* culture every 2 months.

These two groups were designed to simulate repeated acute exacerbations of chronic pyelonephritis

Group B was not given any additional injections. These animals were considered to have simple chronic pyelonephritis.

Group C consisted of the uninfected control animals.

Blood pressure determinations were made by a modification of the tail plethysmographic method described by Williams, Harrison, and Groll-

(2) The enterococcal populations of both cortices were the same during the first day. Thereafter, the number of bacteria in the cortex of the obstructed kidney increased more rapidly than that of the normal cortex

(3) Although the changes in microbial population in the whole normal and obstructed kidneys were similar between the sixth hour and the fourteenth day after infection, the number of bacteria in the obstructed organ was somewhat higher. After the fourteenth day the population in the obstructed kidney declined, apparently as a result of the unfavorable environment created by the accumulation of pus and the products of tissue destruction. During this time, the bacteria in the normal kidney persisted in relatively constant numbers.

Pathology of Lesions Produced by Streptococcus faecalis *in the Rat*

Macroscopic and microscopic observations were made of the organs of animals at time of sacrifice. The only pathologic changes observed in the liver were small perivascular collections of mononuclear cells first seen 2 to 3 days after injection. This process subsided by the seventh to tenth day without apparent residua. In the spleen, moderate follicular hyperplasia was noted during this period but tended to subside by the tenth day. An occasional animal examined after this period had evidence of splenic hyperplasia, but these changes were confined to rats with acute exacerbations of chronic renal infection. The heart was examined in approximately two-thirds of the animals studied in these experiments In no instance was endocarditis, pericarditis, or myocarditis noted.

The earliest macroscopic lesions seen in the kidney were discrete or confluent cortical abscesses measuring 1 to 3 mm. in diameter. These were first observed in some animals by the fifth day. Thereafter, the frequency of occurrence of gross renal lesions increased as a function of time, so that almost all of the kidneys examined after the sixth week had anatomic evidence of infection. Although abscesses persisted in some rats up to 4 weeks after inoculation, evidence of healing became apparent by the fourteenth day in the majority of animals and was manifested by diminution in the amount of pus and beginning contraction of the involved tissues At 6 weeks, scars were seen on the surface of the kidney in most animals. These were irregular in shape, depressed, and had flat "U-shaped" bases. As the experiments progressed, occasional instances were noted in which acute abscesses were found adjacent to areas of scar tissue This corresponded with some increase in the bacterial population of the kidney and was interpreted as representing acute exacerbations of chronic pyelonephritis

Although the earliest macroscopic lesions were noted 5 days after injection, microscopic changes occurred before this time. At 2 days, small focal collections of leukocytes were seen in the interstitium of the

Despite frequently noted extensive evidence of infection, no vascular alterations were observed in chronic enterococcal pyelonephritis in the rat. Similarly, Shapiro *et al.*[8] and Heptinstall and Gorrill[7] did not find vascular changes in their animals. This lack of blood vessel pathology might account for the infrequent occurrence of hypertension in the models cited. The development of such vascular alterations may be dependent on type and severity of infection, animal species, and other factors.

Summary

A model of chronic, progressive enterococcal pyelonephritis in the rat has been described. This infection is characterized by the persistence of bacteria in relatively constant numbers during the evolution of the disease from the acute to the chronic stage.

A comparatively large number of bacteria was required to cause infection. It was estimated that the kidney-infecting dose (K.I.D. 50) necessary to produce pyelonephritis in 50 per cent of animals tested was 10,000 viable enterococci.

Quantitative observations were made on the microbial populations in the cortex and medulla at various intervals after intravenous injection of bacteria, and evidence was presented which indicated that the medulla was the part of the kidney most susceptible to infection. It was also in this area that the earliest histologic evidences of infection were noted.

While no increased "trapping" of microorganisms was noted in the kidney with ureteral obstruction when this organ was cultured as a whole, studies of the various renal areas indicated that ureteral ligation did result in a greater bacterial population in the medulla than was found in the medulla of the normal kidney. This difference was noted as early as 30 minutes after injection and persisted throughout the period of observation (5 days). In the cortex, similar numbers of bacteria were found during the first day after inoculation. Thereafter, the population in the cortex of the kidney with ureteral obstruction increased more rapidly than that of the normal cortex. These results emphasize the importance of considering the cortex and medulla as separate tissues in studies of the host-parasite relationship.

Several strains of *Str. faecalis*, *Str. faecalis* var. *zymogenes*, and *Str. faecalis* var. *liquefaciens* produced renal infection in the majority of rats injected. A single culture of *Str. durans* failed to cause infection in any of the animals tested.

Blood pressure recordings were obtained in groups of rats with chronic pyelonephritis and chronic pyelonephritis with acute exacerbations of enterococcal or *E. coli* infection. Observations were made for 12 months

of *E. coli* in the normal medulla and in the medulla of the kidney in which ureteral obstruction had been performed.

The failure to demonstrate bacteriuria in the first 2 days after intravenous inoculation despite the presence of large numbers of microorganisms in the kidney and the appearance of enterococci in the urine coincidentally with the appearance of microabscesses supports the hypothesis that the kidney does not function as a filter of bacteria.[2] Tissue damage must occur in order to permit bacteria to escape into the tubular collecting system and appear in the bladder urine. The lack of evident cystitis in animals with chronic pyelonephritis and significant bacteriuria suggests a remarkable protective ability of the intact bladder mucosa.

The results obtained in the experiments performed in an attempt to determine the number of bacteria required to produce pyelonephritis indicated a direct relationship between inoculum size and percentage of animals infected. When this was considered with the data pertaining to the microbial population in the kidney shortly after intravenous injection, it was determined that the infecting tissue population which caused pyelonephritis in 50 per cent of animals was 10,000 viable enterococci This may be compared with a kidney-infecting dose (K.I.D. 50) of between 1000 and 10,000 staphylococci required to produce pyelonephritis in the normal mouse[5] Using this kind of data, it would be of interest to determine the fate of subinfective inocula within the cortex and medulla in order to understand better the renal-host resistance mechanisms.

The failure to observe hypertension in chronic enterococcal pyelonephritis was similar to the experience of Shapiro, Braude, and Siemienski,[6] who studied blood pressure changes in the rat with chronic, nonobstructive pyelonephritis produced by injecting gram-negative bacteria into animals which had been "prepared" by renal massage. No significant blood pressure changes were noted in groups of animals observed for 30 and 42 weeks Heptinstall and Gorrill[7] induced renal infection in rabbits in which one ureter had been ligated. In the presence of unilateral pyelonephritis and intact contralateral kidney no blood pressure changes were noted. When the uninfected kidney was removed, 5 of 27 animals developed blood pressure increases ranging from 16 to 57 mm. Hg. Spitznagel and Schroeder[8] produced pyelonephritis in the rat by intravenously injecting *E. coli* into animals with unilateral ureteral obstruction. A significant number of animals developed hypertension when studied for periods up to 127 days. The authors stated that "Vascular changes in the right unoperated kidney were observed in the animals with hypertension of several weeks duration. This was usually less in degree from that observed in the infected kidney, but was easily demonstrable. In only one animal was it marked." The exact nature of these vascular changes was not described.

3

*Experimental Pyelonephritis Consequent to Induction of Bacteriuria**

E. VIVALDI, M.D., D. P. ZANGWILL, M.D., R. COTRAN, M.D., and E. H. KASS, M.D., Ph.D.

(Boston, Massachusetts)

Experimental pyelonephritis has generally been produced by methods involving intrarenal or extrarenal obstruction to the flow of urine with the subsequent intravascular injection of bacteria. Ureteral ligation,[6, 7, 8] electrocauterization of the renal medulla,[10] renal massage,[3] or the scars of antecedent staphylococcal infection[2] have resulted in the localization of bacteria in the traumatized kidney with the subsequent development of acute pyelonephritis. In addition, the intravascular injection of certain bacteria produces acute pyelonephritis in different animal species in a varying proportion of animals.[2-4, 6, 8]

In the systems in which gram-negative rods have been used, the bacteria generally disappear from most of the kidneys within a few weeks, the inflammatory process either heals, with residual scarring, or it destroys the kidney within a short period of time. The experimental production of chronic active pyelonephritis or of hypertension has not been clearly demonstrated in such systems, and only healed lesions have usually been found. When enterococci or staphylococci have been given, interstitial abscesses have been produced, but hypertension has not developed and the morphologic lesions have not been directly comparable to those of chronic active pyelonephritis in man.

The importance of bacteriuria in the pathogenesis of urinary tract infections has been demonstrated in our laboratories and will be reviewed elsewhere during this symposium by Kass. Suffice it to say that there is increasing clinical evidence that bacteriuria predisposes to pyelonephritis.

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ments of Pathology, Medicine, [illegible]
School, Boston. Aided by gra[illegible]
States Public Health Service, t[illegible]
Foundation.

after onset of infection and during this time no instances of significant, sustained hypertension were noted.

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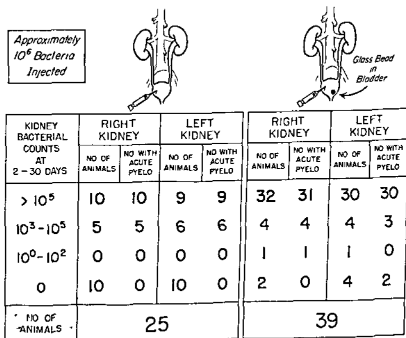
which bacteriuria was induced developed typical bilateral acute pyelonephritis. Within 24 hours after bacteriuria had been induced, more than 50 per cent of the animals had bacteria in the kidney although morphologic evidence of pyelonephritis had not yet developed in all of them. After 24 hours, the presence of bacteria in the kidney is associated with morphologic evidence of acute pyelonephritis in about 98 per cent of instances. In those animals in which a glass bead was inserted into the bladder, the incidence of bacteria in the kidney and of pyelonephritis rose. The inflammatory process in the kidney was more severe in animals with glass beads. The acute mortality rate during the first week was approximately 60 per cent compared with a mortality rate of about 10 per cent in the animals without a glass bead in the bladder.



FIGURE 2 Gross appearance of kidney of a rat sacrificed 4 days after induction of bacteriuria. Many individual and confluent abscesses are visible on the cortical surface.

Therefore, we studied the relationship of induced bacteriuria to pyelonephritis White rats weighing between 150 and 200 Gm were anesthetized with ether, the bladder was exposed through a suprapubic incision, and approximately one million bacteria were injected into the bladder lumen In some groups of animals a smooth glass bead, about 3 mm. in diameter, was placed in the bladder prior to the injection of bacteria The test organism was a strain of *Proteus vulgaris* grown overnight in nutrient broth. Control animals were submitted to the same surgical procedure, but without the injection of bacteria. Animals were sacrificed at various time intervals from 1 day to 5 months after surgery. At the time of sacrifice, blood cultures were taken from the heart with the animal under light ether anesthesia. The kidneys were then removed aseptically through separate flank incisions and divided under sterile precautions The bacteria in one half kidney were counted by homogenizing the kidney in sterile broth and by performing serial pour plate dilutions The other half was fixed and sectioned for morphologic study. Sections were also made of bladder, ureter, and other organs. Bladder urine of each animal was cultured.

Figure 1 summarizes the data obtained in animals sacrificed between 2 and 30 days after the induction of bacteriuria. Fifteen of 25 animals in



KIDNEY BACTERIAL COUNTS AT 2 – 30 DAYS	RIGHT KIDNEY		LEFT KIDNEY		RIGHT KIDNEY		LEFT KIDNEY	
	NO OF ANIMALS	NO WITH ACUTE PYELO	NO OF ANIMALS	NO WITH ACUTE PYELO	NO OF ANIMALS	NO WITH ACUTE PYELO	NO OF ANIMALS	NO WITH ACUTE PYELO
$> 10^5$	10	10	9	9	32	31	30	30
10^3-10^5	5	5	6	6	4	4	4	3
10^0-10^2	0	0	0	0	1	1	1	0
0	10	0	10	0	2	0	4	2
NO OF ANIMALS	25				39			

FIGURE 1 Production of acute pyelonephritis in rats Results subsequent to the intravesical inoculation of *Proteus vulgaris*

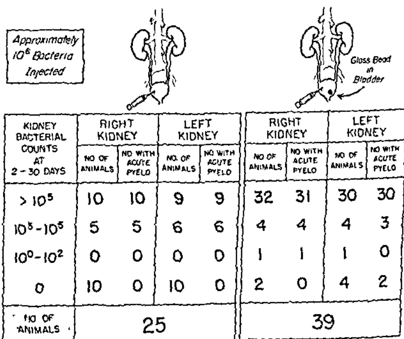
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KIDNEY BACTERIAL COUNTS AT 2 – 30 DAYS	RIGHT KIDNEY: NO OF ANIMALS	RIGHT KIDNEY: NO WITH ACUTE PYELO	LEFT KIDNEY: NO. OF ANIMALS	LEFT KIDNEY: NO WITH ACUTE PYELO	RIGHT KIDNEY: NO OF ANIMALS	RIGHT KIDNEY: NO WITH ACUTE PYELO	LEFT KIDNEY: NO OF ANIMALS	LEFT KIDNEY: NO WITH ACUTE PYELO
$> 10^5$	10	10	9	9	32	31	30	30
10^3-10^5	5	5	6	6	4	4	4	3
10^0-10^2	0	0	0	0	1	1	1	0
0	10	0	10	0	2	0	4	2
NO OF ANIMALS	25				39			

FIGURE 1. Production of acute pyelonephritis in rats. Results subsequent to the intravesical inoculation of *Proteus vulgaris*



Figure 2 shows the gross appearance of the kidney of an animal autopsied on the fourth day after induction of bacteriuria.

Figure 3 shows that the lesion is characteristic of acute pyelonephritis. The glomeruli, in general, are unaffected by the acute process. The induction of bacteriuria produces acute pyelonephritis in this experimental model in the absence of injury or obstruction to the kidney or ureter.



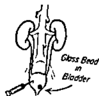
FIGURE 3. Microscopic appearance of kidney of a rat sacrificed 4 days after induction of bacteriuria There is interstitial infiltration with polymorphonuclear leukocytes

In control animals, without injection of bacteria into the bladder, bacteriuria due to the surgical procedure rarely occurs (Figure 4). The incidence of acute pyelonephritis in control animals is 4 per cent.

The next experiments were designed to determine whether bacteriuria leads to pyelonephritis by ascent of bacteria via the ureter to the kidney, or by localization of bacteria in the kidney after bacteremia secondary to invasion of the bladder.

Figure 5 shows the results in animals sacrificed 24 hours after inoculation of *bacteria into the bladders* In these animals, bacteria are found in 60 per cent of the kidneys. After double ligation and interruption of one ureter a [illegible] [illegible]tion of bacteriuria, 50 per cent of kidneys with an intact ureter h[illegible] [illegible] bacteria, generally in high numbers In those kidneys with the [illegible] the[illegible] only 9 per cent had viable bacteria and the numbers [illegible]





KIDNEY BACTERIAL COUNTS AT 4–30 DAYS	RIGHT KIDNEY		LEFT KIDNEY		RIGHT KIDNEY (Glass Bead in Bladder)		LEFT KIDNEY (Glass Bead in Bladder)	
	NO OF ANIMALS	NO WITH ACUTE PYELO	NO OF ANIMALS	NO WITH ACUTE PYELO	NO OF ANIMALS	NO WITH ACUTE PYELO	NO OF ANIMALS	NO WITH ACUTE PYELO
$> 10^5$	0	0	0	0				
10^3-10^5	1	1	1	1	2	2	2	2
10^0-10^2	1	1	0	0	1	1	0	0
0	25	0	26	0	0	0	0	0
					74	0	75	0
NO OF ANIMALS	27				77			

Figure 4 Production of acute pyelonephritis in rats Results subsequent to the intravesical inoculation of sterile broth

Kidney Bact Counts at 24 hrs.	RIGHT KIDNEY	LEFT KIDNEY	RIGHT KIDNEY	LEFT KIDNEY	RIGHT KIDNEY	LEFT KIDNEY
$>10^5$	34%	34%	26%	0	10%	48%
10^3-10^5	0	3%	12%	0	24%	5%
10^0-10^2	25%	18%	12%	9%	57%	42%
0	41%	45%	50%	91%	9%	5%
No of Rats	29		34		[illegible]	

Figure 5 Demonstration of ascending infection in rats [illegible] after [illegible] to the production of bacteriuria.

of bacteria were lower than on the other side. Statistical analysis revealed that the difference between the sectioned and intact sides was significant at a level of confidence exceeding 99 per cent. The presence of bacteria in kidneys with interrupted ureters was almost always associated with bacteremia.

For these data to indicate ascension of the infection, it must be shown that the unobstructed kidney is not more susceptible to hematogenous infection than is the obstructed kidney. This is demonstrated in the third column of Figure 5. No interpretation is possible other than that infection of the kidney ascends via the ureter to the kidneys in this experimental system. We do not yet know whether this ascent is via the lumen or in the interstitial pathways. *The experiment indicates that the production of bacteriuria leads to the development of acute pyelonephritis by means of an ascending mechanism.* This would indicate that persistent chronic active pyelonephritis could be produced by constant reinfection of the kidney.

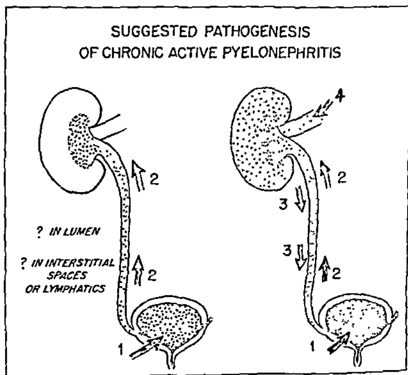
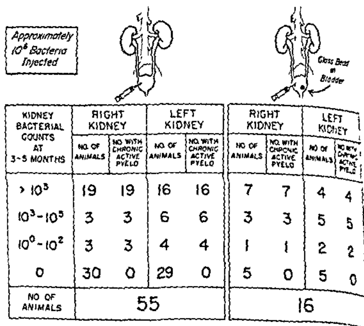


FIGURE 6 Suggested pathogenesis of chronic active pyelonephritis. Bacteriuria is induced (1), and the bacteria reach the kidney via the ureter (2). Bacteria spread within the kidney and are subsequently discharged into the urine (3), maintaining the bacteriuria. The cycle may be initiated by hematogenous spread of bacteria to the kidney (4).

Figure 6 describes this concept. On the left side, bacteriuria is [illegible] and the infection reaches the kidney by way of the ureter. On the [illegible] side of the diagram, the infection has spread throughout the [illegible]

cycle.

chronic active pyelonephritis.



KIDNEY BACTERIAL COUNTS AT 3-5 MONTHS	RIGHT KIDNEY		LEFT KIDNEY		RIGHT KIDNEY		LEFT KIDNEY	
	NO. OF ANIMALS	NO. WITH CHRONIC ACTIVE PYELO	NO. OF ANIMALS	NO. WITH CHRONIC ACTIVE PYELO	NO. OF ANIMALS	NO. WITH CHRONIC ACTIVE PYELO	NO. OF ANIMALS	NO. WITH CHRONIC ACTIVE PYELO
$>10^5$	19	19	16	16	7	7	4	4
10^3-10^5	3	3	6	6	3	3	5	5
10^0-10^2	3	3	4	4	1	1	2	2
0	30	0	29	0	5	0	5	0
NO. OF ANIMALS	55				16			

Blood cultures obtained at the time of autopsy were free of proteus in all animals.

FIGURE 7 Production of chronic active pyelonephritis in rats subsequent to the intravesical inoculation of *Proteus vulgaris*.

pyelonephritis, azotemia was present, as evidenced by the mean blood urea nitrogen of 51 mg per cent, with 40 per cent of the animals having a value above 40 mg. per cent.

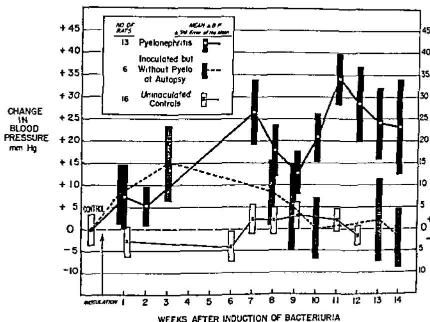


FIGURE 10 Effect of chronic active pyelonephritis upon systolic blood pressure The systolic blood pressure rose above 150 mm. Hg in about one-half of the animals with morphologic evidence of chronic active pyelonephritis

In conclusion.

(1) The induction of bacteriuria with *P. vulgaris* produces pyelonephritis consistently in the rat without demonstrable obstruction of the urinary tract.

(2) The pyelonephritis so produced ascends from the bladder to the kidneys via the ureter.

(3) The pyelonephritis so produced becomes chronic, with bacteria persisting in the kidneys for several months. The lesion is associated with a relatively high rate of induction of systolic hypertension and of azotemia.

(4) It is suggested that chronic active pyelonephritis is due to the continued reinfection of the kidney by ascending infection from the bladder.

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Materials and Methods

Female Holtzman strain white rats weighing 200 to 250 Gm. were inoculated with 1 ml. of varying dilutions of an 18-hour broth culture of various *Escherichia coli* serotypes or enterococcus species. Inoculation was performed in ether-anesthetized animals by injection through a PE #50 polyethylene catheter inserted into the urinary bladder, or alternatively through a needle inserted into the heart or great vessels Routinely the right kidney was massaged through the intact abdominal wall for 5 minutes after either method of inoculation.

The bacterial count of the initial 18-hour broth culture was determined by nephelometer approximation, and appropriate dilutions were made to obtain the desired inoculum size. The culture was then refrigerated until the termination of the injection period. Serial tenfold dilution plate counts were used for verification of the bacterial count.

All bacterial strains had been isolated from patients with urinary tract infections. After initial isolation and biochemical determinations, the strains were kept refrigerated on agar slants until immediately prior to their use. All strains had been kept for several months Single colony isolates were tested for their antigenic composition at the Communicable Disease Center, Chamblee, Georgia, through the courtesy of Dr. W. H. Ewing

The pathogenicity of strains of *E. coli* was tested with regard to the following characteristics motility, surface antigens (K), somatic antigens (O), and the fermentation reaction with salicin or sucrose.

For the most part, 3 animals were sacrificed 30 minutes, 4 hours, 24 hours, and 72 hours following injection. Three additional rats were sacrificed each week for 12 weeks Some animals were kept at least 6 months following a single intrabladder injection before sacrifice.

The animals were killed with chloroform or ether and dipped in phenol solution, the abdominal cavity was opened aseptically. Twenty-five hundredths of a milliliter of blood was aspirated from the heart and cultured in tryptose phosphate broth. Both kidneys and the spleen were removed and bisected. The renal pelvis and medullary tissue was separated from the cortical parenchyma of one-half of each kidney and the specimens dropped into 4.5 ml of broth for bacterial culture. This represents approximately a 1:10 weight/volume dilution. The entire urinary bladder was removed and cultured similarly. After an incubation period of 4 hours which was assumed to represent the bacterial lag phase during which diffusion of bacteria, tissue fluids, and broth would occur, a standard aliquot (.01 ml.) was plated on eosin-methylene blue agar and the number of colonies enumerated after 24 hours incubation. The broth tube containing the organ under examination also was incubated and inspected for

gross turbidity from bacterial growth after 24 hours. In the absence of obvious growth a standard aliquot was streaked onto an agar plate and incubated. The original organ was then continued under incubation for 2 weeks or longer and inspected periodically for bacterial growth. This method would theoretically reveal bacteria in excess of 2 per kidney. Approximately one of each fifteen of the kidneys was randomly selected for histologic sections.

Results

Preliminary studies performed with a strain of enterococcus and one strain of *E. coli* demonstrated a reproducible pattern of definite stages in the natural course of the bacterial infection in the kidneys. These are illustrated in Figures 1, 2, and 3. Figures 1 and 2 are presentations of the

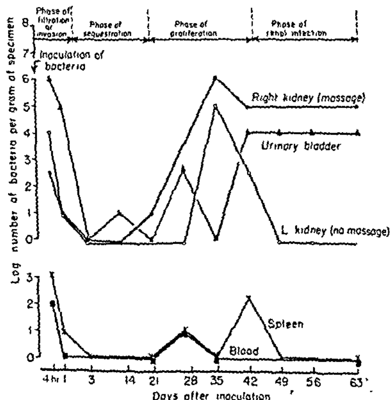


FIGURE 1 Bacterial counts in the blood, spleen, and urinary tract following the inoculation of enterococcus species into the bladder

lesions were observed in some specimens within 72 hours after injection by either route

Phase of Bacterial Sequestration

Following the initial phase, there is a period from three days to four to six weeks in which no bacteria, or only a very few, could be cultured from the kidneys, urinary bladder, spleen, or blood. During this period of apparent bacterial sequestration, some gross and microscopic renal and bladder lesions were demonstrable, despite the inability to culture microorganisms.

Attempts to demonstrate L-form variants during this phase were unsuccessful either by culture of the renal tissue on special solid agar or by regrowth of bacteria in fresh broth after tissue homogenization.

Agglutinating antibodies against the infecting bacterial strain were absent from the blood of the animals one week after infection, whereas high agglutinating titers (80 to > 320) were uniformly present five weeks after infection.

Phase of Bacterial Proliferation

Four to six weeks following the initiation of infection, bacterial proliferation appeared to occur and progressively larger numbers of bacteria (10^6) could be isolated from the kidney. This phase was delayed in development, less intense, and more transient in the unmassaged kidney but nevertheless readily demonstrable in either kidney. Occasionally similar changes were observed in the spleen with or without a return of bacteremia. This phase merges into the alternative courses of the fourth phase.

Phase of Chronic Renal Infection or Remission

A continued infection of the kidney evidenced by consistent recovery of large numbers of bacteria (10^5) and histologic signs of active renal infection were found in some specimens for at least six months. Alternatively bacteria were recovered in progressively smaller numbers from some specimens and finally not at all, as shown in Figure 2. This disappearance of bacteria may represent cure, but this is not certain since microscopic inflammatory lesions were observed in some of these kidneys

During the last two phases, agglutinins for the infecting strains persisted at the level of approximately 1:320 or greater. This finding did not offer any distinction between animals whose organs harbored chronic infection and those that did not.

Microscopic lesions were observed during the phase of filtration or invasion Large numbers of polymorphonuclear leukocytic tubular casts were present (Figure 4). Interstitial round cell infiltration occurred as focal inflammatory lesions in both the medulla and cortex (Figure 5) or



FIGURE 4. Photomicrograph of rat kidney demonstrating polymorphonuclear leukocytic tubular casts during the phase of filtration and invasion. Photograph was made from an unmassaged kidney of a rat infected by the intrabladder injection of *Escherichia coli*.



[illegible]

bladder catheterization. It was not possible to culture bacteria from this kidney.

as wedge-shaped areas of heavy infiltrate extending from the medulla into the cortex. Periglomerular and periarterial inflammatory lesions were observed without definite evidence of involvement of the glomerulus or the artery (Figure 6). Similar changes were observed during the phases of sequestration, proliferation, and chronic infection, except that the cellular tubular casts were found only in the early phases of infection.

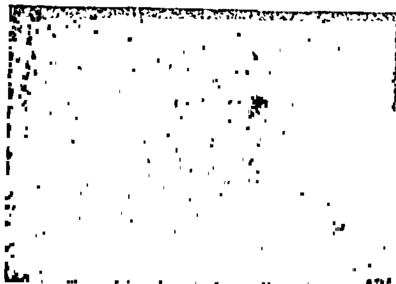


FIGURE 6 Photomicrograph of a section of rat kidney infected with *Escherichia coli* intravascularly There is an area of round cell infiltrate about a glomerulus and artery without apparent involvement of either of these structures

Further studies were done to test the importance of various biochemical and serologic differences among strains of *E. coli* upon the course of retrograde infection and to compare them with enterococci Distinctly lower bacterial counts were obtained from the kidneys during the phases of proliferation and chronic infection in this group of experiments, but all of the previously described phases of renal infection could be readily identified.

A total of 738 rats survived longer than 24 hours after the original infection The over-all fatality rate within the first 24 hours was 6 per cent. Another 12 per cent of the rats died later during the experiments but before sacrifice. No attempt was made to study fatalities bacteriologically or microscopically.

Pyelonephritis was observed in 32 per cent of the examined kidneys were sacrificed animals. Table I shows the results of infections produced focal inflammato onmotile bacterial strains with otherwise similar antigen

TABLE I THE FATALITY RATE AND INCIDENCE OF HISTOLOGIC PYELONEPHRITIS IN THE MASSAGED AND UNMASSAGED KIDNEYS OF RATS INFECTED WITH *Escherichia coli* STRAINS WITH VARIOUS ANTIGENIC CHARACTERISTICS

Escherichia coli Strain Characteristic	No of Animals	Died		Sacrificed			
				Kidney Massaged		Kidney Not Massaged	
		Early (per cent)	Late (per cent)	No Obs	Histologic Pyelonephritis (per cent)	No Obs	Histologic Pyelonephritis (per cent)
Motile	192	10	15	25	48	23	17
Nonmotile	144	4	5	23	39	26	27
Surface antigen							
K (L)	192	7	13	27	41	27	15
K (A)	192	10	17	23	39	21	23
K-negative	192	1	2	34	35	35	29
Somatic antigen							
O Pool I	240	6	10	30	33	31	16
O Pool II	240	10	13	33	43	31	26
O Pool III	96	3	3	21	38	22	27

composition Motile strains produced more acute (10 per cent) and late (15 per cent) deaths than did nonmotile strains (4 and 5 per cent). The incidence of microscopic renal lesions in both kidneys was 33 per cent with each strain No difference existed between the incidence of lesions in the massaged and unmassaged kidney with nonmotile strains, but motile strains produced histologic pyelonephritis significantly more frequently ($p < .02$) in the massaged kidney than its unmassaged mate

Studies pertaining to surface antigens are also summarized in Table I. *E coli* possessing K (A) antigens produced more acute (10 per cent) and late (17 per cent) deaths than did organisms with K (L) antigens, and the latter caused more deaths than *E coli* without surface antigens. The total incidence of histologic pyelonephritis was similar regardless of surface antigens, but K (L) strains had significantly greater localization ($p < .05$) in the massaged kidney and strains possessing no surface antigens caused an almost equal frequency of lesions in the massaged and unmassaged kidneys

Strains of *E coli* with somatic (O) antigens represented in one of three major pools of similar serologic types were tested, the results are shown in Table I Animals infected with *E coli* from somatic antigen pool II (O 11, O 19) produced more acute and late deaths than the strains from pool I (O 25, O 6, O 7) The strains with (O) antigens in pool III (O 22) produced only 3 per cent acute and late deaths The differences in the incidence of microscopic pyelonephritis among these strains ... per cent, were not significant. Strains with somatic antig...

as wedge-shaped areas of heavy infiltrate extending from the medulla into the cortex. Periglomerular and periarterial inflammatory lesions were observed without definite evidence of involvement of the glomerulus or the artery (Figure 6). Similar changes were observed during the phases of sequestration, proliferation, and chronic infection, except that the cellular tubular casts were found only in the early phases of infection.

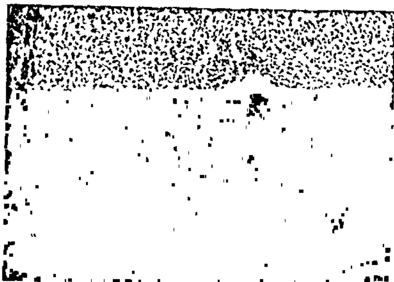


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GENERAL DISCUSSION

Dr. Beeson. We are now ready for discussion (Pause.) Since there is none from the floor, Dr. Kass, will you open the discussion?

Dr. Kass The pattern that has emerged is so clear, and the problems that the experimental approaches present so obvious, that we all share a feeling of humility about the field.

Among the questions that one could phrase would be What speculations could account for the difference in the effect of medulla and cortex in supporting bacterial multiplication?

I realize this is jumping the gun a little on some of the work that will be presented later, and particularly the work that you and your associates have done, Dr. Beeson I think also that we ought to recognize the initial experiments that you and your associates, Dr Guze and Dr. Freedman, have performed in indicating the special capacity of the medulla to support bacterial multiplication So I should like to ask both Dr. Gorrill and Dr Guze to formulate, on admittedly hypothetical grounds, various explanations of this extraordinary difference between cortex and medulla in supporting bacterial multiplication.

Dr Gorrill I am afraid I would not be able to cast any light on this particular problem It is one which I agree is at the root of the matter.

I feel in some ways that a more useful approach to the problem is to say to oneself, "Why don't all bacteria multiply all the time in the kidney?" As far as I know, the kidney has no antibacterial system apart from the mechanism which I believe you have been uncovering recently. The kidney would appear to be wide open to infection. A number of workers with other bacteria have shown that chronic infection of the kidney may persist for months. We have been able to show it. Why is it that the kidney resists infection at all? Perhaps I should cast the ball back and someone might tell us why he thinks the normal kidney with no trauma and no damage is infected repeatedly.

Dr. Guze I should like to add some comments Although we did not present some of our data at this meeting, the differential behavior of infection in the cortex and medulla is even more striking if one considers the response of this model to therapy. In the untreated animal, evidence has been obtained which suggests that after a while the bacterial population in the cortex "burns out" and the infection persists in the medulla

During the course of antibiotic treatment, this difference in the behavior of bacteria in the cortex and in the medulla is even more striking

potential of the medium by the 99 per cent that died off. If glutathione was added at the same time, all the bacteria grew.

DR. NOVIKOFF: You all know that in the medulla there is an abundant interstitial substance; this afternoon I will demonstrate by slides that it contains a metachromatic material in which specialized interstitial cells are arranged in characteristic fashion. The metachromatic material may provide a milieu in which the bacteria may grow. If present at all in the cortex, its concentration is too low to be demonstrable by the staining methods employed.

A slide I will show bears on the phagocyte question. It is a section of kidney from the so-called Gunn rat, a strain in which little bilirubin is transferred by the liver cells into the bile. Bilirubin accumulates in the renal papilla, particularly at the apex. Throughout the papilla there are these cells with large cytoplasmic bodies (lysosomes) containing acid phosphatase. The cells resemble the interstitial calls of normal rats, but in this kidney they possess the large lysosomes characteristic of phagocytes.

I would suggest that the Gomori technique for acid phosphatase, when applied to frozen sections of formal-calcium-fixed tissue, is excellent for demonstrating phagocytes, even those which escape ready detection in hematoxylin-eosin preparations.

DR. MERRILL: I should like to ask Dr. Vivaldi if he found any difference in the morphology which would account for the fact that his *Proteus* animals developed hypertension, whereas the animals of the enterococcal group did not develop hypertension. Were there any vascular lesions, or was it an overwhelming infection?

DR. BEESON: Dr. Kass, would you care to reply to Dr. Merrill's question?

DR. KASS. Several points of importance have arisen, and I should like to comment briefly on a few of them.

First, I should like to make the general point that we have two different philosophies of approach going on here, and I think we ought to try to keep them separated to some degree. On the one hand there is the attempt to understand human pyelonephritis, using the animal model On the other there is the attempt to look more deeply into the general problem of host-parasite relationships, using renal infection as a model These are equally valid approaches to any problem, but they are separable to some degree. To put these two together prematurely may not serve us particularly well.

Our approach in the paper Dr. Vivaldi presented has been aimed at obtaining a model of human pyelonephritis based on observations in the

human disease Out of it perhaps may emerge other aspects that will be relevant to the general problem of the host-parasite relation. This is different from starting with, let us say, an enterococcic system and determining what happens to bacteria which perchance light in the kidney, because the kidney has a very special quality that permits bacterial multiplication—a problem of great fascination which may or may not be relevant to the question of what happens in most human pyelonephritis.

This is relevant also to the choice of *Proteus* as the experimental organism in our experiments It has been customary in the field of experimental pyelonephritis to start almost every paper with the sentence, "An organism was obtained from a patient who was seriously ill with pyelonephritis . . ." We were studying the rat. The organisms obtained from a human infection may or may not be pathogenic for the rat. There is no compulsion that they be so. So we started with the rather foolish assumption that there might be organisms pathogenic for rats regardless of whether or not they were pathogenic for man *Proteus* is the natural pathogen for rats. In experiments in which *Escherichia coli* was instilled into the bladders of rats, with a glass bead to help the organisms to remain there for a while, there was a high likelihood that the animals with the *E. coli* would spontaneously acquire *Proteus* infection and develop disease exactly like the one produced experimentally with *Proteus* alone

The pathologic findings are of major importance, and time did not permit a detailed discussion After about four months of chronic *Proteus* bacteriuria in the rat, the kidney is one-half to two-thirds the usual size of a kidney of a rat of this age and weight. There are many scars and the kidney is shrunken and contracted The sections taken from these kidneys show chronic pyelonephritis with foci containing polymorphonuclear leukocytes. So I think we have here the first experimental model that closely resembles active, progressive chronic pyelonephritis in man

With respect to the matter of trauma and what happens when the kidney is or is not traumatized by massage, I would point out that in Dr. Braude's technique five minutes of massage, however lightly, was specified. If one does the same experiment without any trauma to the kidneys, as he has recently published with his associates, one nevertheless can reproduce chronic *Proteus* infections after intravenous inoculation. In our hands about 18 per cent of the rats so injected developed frank pyelonephritis, bacteriuria commonly occurred, and the cycle was instituted. If a little glass bead was put into the bladder just to help the bacteriuria persist, the incidence of pyelonephritis rose to 50 per cent after intravenous injection of *Proteus*

With respect to Dr Merrill's most important question on hypertension, I wish we knew the answer One can merely suggest that the difference between the *Proteus* system and the enterococcic system is the possibility

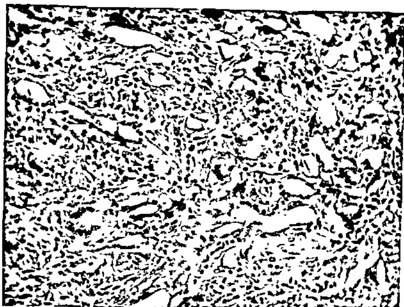


FIGURE 4. Higher enlargement of granuloma shown in Figure 3 Foreign body granuloma containing sulfonamide crystals H & E ×150

entirely lacking in the contralateral organ. In one of these cases the precipitates had caused the development of large nodular foreign body granulomata. Probably the glomerular filtration of this nonfunctioning kidney was relatively well maintained, whereas the distal pyelonephritic changes were so severe that the glomerular filtrate was totally reabsorbed except for the sulfonamides which precipitated.

With Geiser[6] we reported that rats given heavy doses of highly insoluble sulfonamides developed genuine chronic interstitial nephritis which was easily distinguishable from chronic pyelonephritis. We shall come back to this differential diagnosis later.

PHENACETIN-CONTAINING ANALGESICS AND PYELONEPHRITIS

To make these connections clear I must make certain introductory remarks and a little detour. In 1950 Spuhler and I[32] pointed out that genuine chronic interstitial nephritis, which in former years was very infrequent, had been noted more and more in the preceding decade The elements of differential diagnosis ... shown
in Figure 5 We con
destructive process,
diffuse manner, particularly at the corticomedullary[31] junction. In this

latter case there are no granulomata but the interstitial tissue shows either a lymphoplasmocellular infiltration, or a predominantly serous inflammation in the acute form. In its chronic form the fibrous interstitial elements are thickened and hyalinized, so that the entire picture resembles an inveterate edema which compresses the parenchymal elements and the

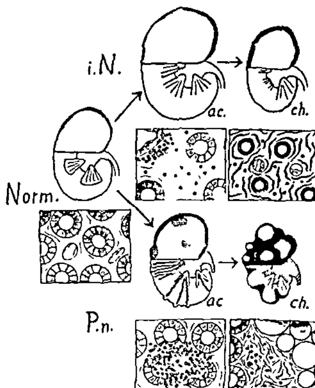


FIGURE 5 Schematic presentation of main differences between normal kidney (left), interstitial nephritis (upper row), and pyelonephritis (lower row), acute and chronic form

vessels but does not destroy them (Figure 6). Pathogenetically pyelonephritis is always due to a local bacterial invasion and multiplication, whereas in acute interstitial nephritis bacterial toxins and/or protein decomposition products damage the organ during their excretion by the kidney.

Concerning the cause of chronic interstitial nephritis, we (1953)[21] considered the possibility that therapy is sufficient in these cases to cause the disappearance of the obvious main symptoms or infective foci, for in-



FIGURE 6 Outer medullary zone of kidney in chronic interstitial nephritis Increase of interstitial tissue with compression of tubules and capillaries, no destruction of parenchyma Infiltration by lymphocytes and plasma cells on the right H & E ×100

stance a tonsillitis or the rash of scarlet fever, whereas the concomitant interstitial nephritis smolders on. That an acute interstitial nephritis may tend to go on into the chronic smoldering form is proven by a recent observation in a little girl who, after a smallpox vaccination, developed an acute interstitial nephritis with anuria. She died in uremia after 45 days on an artificial kidney. Histologic findings were those of typical chronic interstitial nephritis (Figures 7 and 8).

On the other hand, early in this work we were struck by the fact that after very thorough questioning, relatively many patients with chronic interstitial nephritis gave histories of abuse of phenacetin-containing analgesic preparations.[25, 33] There is an enormous abuse of these compounds in Switzerland[10, 18] According to Moeschlin[18] this seems also to be true for the United States, since the average consumption of phenacetin in both lands is said to be 22 Gm. per person per year. Similar terrifying statistics have been reported by Larsen and Møller in Denmark.[12] Of 698 patients examined, approximately 30 per cent took phenacetin daily. Every seventh patient in this series had consumed phenacetin for the preceding six years or longer. In 33.2 per cent of the phenacetin consumers the kidney function was reduced, as compared with only 8.6 per cent of the control group. Many Swiss and some Danish authors have confirmed the con-

nection between chronic abuse of phenacetin and chronic interstitial nephritis[5, 7, 8, 12, 15–17, 19, 21–23, 25] A symposium on phenacetin abuse and kidney lesions held in Freiburg in 1958 came to similar conclusions[21]

On the other hand, it must be stated that an absolute connection between phenacetin abuse and chronic interstitial nephritis does not exist,



FIGURE – Subacute interstitial nephritis in a 9-year-old girl (see text) General increase of interstitial tissue particularly in the corticomedullary junction PAS ×5

since in some cases of this disease a phenacetin abuse can be definitely disproven, and conversely most phenacetin abusers do not develop it. In animal experiments the feeding of phenacetin-containing compounds does not alone suffice to produce kidney changes. In rabbits which had been fed 2 tablets of Saridon, containing 0.5 Gm. of phenacetin, daily for one year, we could not detect any morphologic kidney lesions though the animals seemed to suffer the same mental torpor as humans who are very heavily addicted to phenacetin Studer and Zbinden[24] in 1955 came to the identical conclusion using rats But such phenacetin-treated animals



FIGURE 8. Higher enlargement of corticomedullary junction of Figure 7. H & E ×150

when exposed to nephrotropic bacteria develop a much more severe and widespread pyelonephritis than do controls without phenacetin.[14, 27]

Of course these experiments do not solve the problem of phenacetin abuse and chronic interstitial nephritis. However, they do point out that the kidneys of animals which have been fed phenacetin are more susceptible to kidney-damaging agents This leads me back after a long detour to my original problem of the relationship between pyelonephritis and phenacetin abuse.

Among 5000 autopsies carried out between 1953 and 1958, we found 407 cases of severe pyelonephritis. Of these, a heavy abuse of phenacetin (more than 5 tablets per day) was found in the case histories of 14. Currently, now that our doctors specifically question patients with pyelonephritis as to their phenacetin consumption, an abuse is finally admitted in nearly 25 per cent of the cases In some of these the clinician felt that the patients had a particularly severe form of the disease and ran an unusually rapid course. But, since pyelonephritis can produce such a wide spectrum of courses, an exact conclusion cannot be established.

On the whole, I would say there is no question about the relationship between the abuse of phenacetin and the increased susceptibility to pyelonephritis. This view is supported by others, for instance Jasinski and Wuhrmann,[11] Uehlinger,[29] and recently Doret and Junod,[5] and Larsen and Møller.[12]

Concerning the pathogenesis of the phenacetin-induced damage to the kidney we have only theories. With Uehlinger,[29] Moeschlin,[15] Gsell,[7] and others, we believe that there may be direct damage to the kidney by phenacetin and/or its decomposition products Furthermore, we have to consider the fact that phenacetin and its breakdown products cause hemolysis and methemoglobinemia Possibly the kidney damage is due to the chronic action of these hemolytic metabolites [11, 16] A purely anoxic lesion by hemolysis, as proposed by Larsen and Møller,[12] we consider highly improbable.

In any case we must make clinicians aware of the connection between phenacetin and pyelonephritis so that in the future, when taking a case history, they will question patients about consumption of the drug A thorough re-examination of the problem in various countries with different habits concerning phenacetin abuse seems urgent.

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6

The Role of Bacterial Urease in the Pathogenesis of Pyelonephritis*

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The bacteria responsible for infections of the kidney have little, if any, capacity for producing infections in other organs. This fact is evident from an analysis of the relative frequency with which different bacterial species are isolated in infected urines. At the Presbyterian Hospital in Pittsburgh, for example, *Escherichia coli* is the most frequent cause of urinary infection and *Proteus* organisms are next (Table I). Then in

TABLE I. Etiology of Urinary Infections in 448 Patients

		Relative Incidence (per cent of total infections)
1	*Escherichia coli*	30.5
2	*Proteus* species	18.4
3	*Aerobacter aerogenes*	16.3
4	*Klebsiella pneumoniae*	10.1
5	Paracolon species	6.3
6	*Pseudomonas aeruginosa*	5.8
7	Enterococci	4.0
8	*Escherichia freundii*	3.3
9	*Alcaligenes faecalis*	2.2
10	*Micrococcus albus*	2.0
11	*Micrococcus pyogenes*	1.1

diminishing order there are *Aerobacter aerogenes*, *Klebsiella pneumoniae*, paracolon organisms, *Pseudomonas aeruginosa*, enterococci, *Escherichia freundii* and *Alcaligenes faecalis*. Although these bacteria are generally regarded as relatively harmless in comparison with *Micrococcus pyogenes* and *Streptococcus pyogenes*, the virulent gram-positive pathogens are

* This work was supported by a grant from the United States Public Health Service (H-3220)

(*a*) *Kidney stones* These were present in 13 of 59 rats (22 per cent) examined in various stages of acute and chronic pyelonephritis.[3, 4] They ranged in size from small concretions of gravel near the papillary tip to large, typical staghorn calculi filling the entire renal pelvis In their

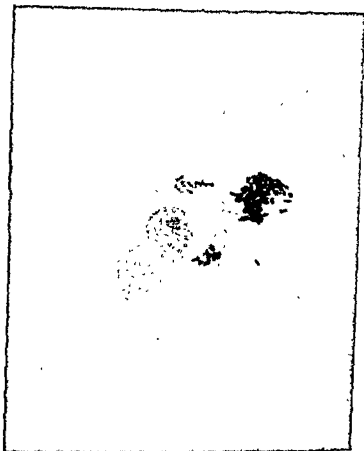


FIGURE 3a Imprint from rat kidney with lesions shown in Figure 2a, 18 hours after inoculation of *Proteus mirabilis*. Note intracellular location of bacterial colonies in cytoplasm of renal epithelial cell (Giemsa ×900).

earliest stages the crystals could be observed as mineral deposits at the tips of the renal papillae and were reminiscent of those described by Randall[5] in human kidneys. On analysis by Dr. E. Prien they were shown to be composed of magnesium ammonium phosphate ($MgNH_4PO_4$), a salt that precipitates in the alkaline environment produced by the accumulation of ammonium after decomposition of urea by *Proteus* urease. Stones

have never been found in numerous rats infected with *E. coli*, *Ps. aeruginosa*, or enterococci, nor in uninfected rats used as controls in these experiments.

(*b*) *Selective intracellular parasitism and necrosis of renal tubules.* The earliest stages of *Proteus* infection of the kidney were characterized by



FIGURE 3b Same as 3a except that bacterial colonies are clustered about nucleus of renal epithelial cell

dense proliferation of the bacteria within tubular epithelium where urea concentration is presumed to be high. Colonies of *P. mirabilis* were observed to localize in the tubular cells within 18 to 24 hours after inoculation (Figure 2a), and stained imprints of individual tubular cells disclosed that the colonies developed in the cytoplasm (Figures 3a and 3b). Infections with staphylococci and *E. coli*, on the other hand, did not

appear to localize or multiply in tubular cells, instead, these bacteria were found outside the tubules and extracellularly (Figures 4 and 5, and reference 2).



FIGURE 4. Section of kidney 18 hours after inoculation of *Micrococcus pyogenes* (H & E ×200). Bacterial colonies (dark staining masses) are situated *between* tubules, but never within the tubules as in Figure 2a. Cellular reaction to *M. pyogenes* has not yet started.

Growth of *Proteus* in tubular epithelium produced necrosis of the tubular cells. By 24 hours these injured tubules were found to be the center of a focal inflammatory reaction beginning with a few polymorphonuclears and later composed of concentric masses of these cells (Figure 2b). This peritubular reaction increased to produce the massive wedge-shaped corticopelvic inflammatory lesions that are characteristic of *Proteus* pyelonephritis[3] In 30 per cent of kidneys, even the early lesion preceding inflammation produced massive gross lesions These pre-

inflammatory lesions had the gross appearance of wedge-shaped areas of yellow necrosis and hemorrhage.

It was suspected that the unique localization of *Proteus* in tubular epithelium was related to a high concentration there of urea (the substrate for



FIGURE 5 Section of kidney 18 hours after inoculation of *Escherichia coli* (H & E. ×180) Bacterial colonies (dark staining masses) are situated *between* the tubules but never within the tubules as in Figure 12

Proteus urease) and that the necrosis resulted from alkalinity secondary to decomposition of urea by the urease. The series of experiments, which follow, were designed to test these possibilities.

Effect of Urea Concentration on Intracellular Infection of Renal Epithelium by Proteus

Tissue cultures of kidney epithelium were better suited than the intact kidney for critically examining the minute details of intracellular infec-

tion by *Proteus*. Confusion between extracellular and intracellular growth was no problem in tissue culture because streptomycin prevented extracellular growth The intracellular growth of *Proteus* in cultures of kidney epithelium 24 hours after inoculation is shown in Figures 6a and 6b The



FIGURE 6a Monkey kidney epithelial cell removed from tissue culture 18 hours after inoculation of *Proteus mirabilis* (Giemsa ×900). Growth of bacterial colony is beginning in cytoplasm of cell Extracellular growth is prevented by streptomycin (50 μgm/ml).

bacteria appeared to grow in well-organized colonies confined to restricted areas of the cytoplasm and presented the same appearance as the cytoplasmic bacterial colonies found in imprints of the renal epithelial cells of pyelonephritic kidneys (Figures 3a and 3b). Their viability was established by obtaining heavy growth upon bacteriologic culture of the infected epithelial cells. As shown in Table II, the per cent of cells in-

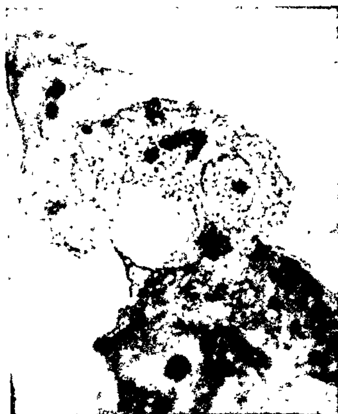


FIGURE 6b Same as Figure 6a showing growth of several colonies within cytoplasm of cell Note absence of extracellular bacteria

TABLE II EFFECT OF UREA CONCENTRATION ON INTRACELLULAR INFECTION OF MONKEY KIDNEY EPITHELIAL CELLS 24 HOURS AFTER INOCULATION OF *Proteus mirabilis* INTO THE TISSUE CULTURE

Concentration of Urea Added to Tissue Culture (milligrams per cent)	Per Cent* of Renal Cells Infected with Colonies of *P. mirabilis*	Per Cent* of Renal Cells Infected with a Few Dispersed Bacteria
100	41	21
50	25	36
0	14	0

* Two hundred kidney cells were counted for each concentration of urea.

fected by colonies of *Proteus* organisms increased as the concentration of urea increased. In other experiments, conducted with higher concentrations of urea, it was found that intracellular growth of *Proteus* was greatest at 200 mg. per cent of urea, a concentration approaching that in the intact kidney. (The average concentration of urea in 12 normal rat kidneys was 201 mg. per cent by weight when determined in kidney homogenates of the whole organ.) The results in Table II indicate that concentrations of urea as low as 50 mg. per cent allowed maximum entrance of single bacteria into cells, but that maximum intracellular bacterial growth in the form of colonies required higher concentrations of urea.

Unlike *Proteus*, inoculation of *E. coli* into tissue cultures produced only slight infection of monkey kidney cells. As shown in Table III,

TABLE III. Effect of Urea Concentration on Intracellular Infection of Monkey Kidney Cells 24 Hours After Inoculation of *Escherichia coli* into Tissue Cultures

Concentration of Urea in Tissue Culture (milligrams per cent)	Per Cent* of Renal Cells Infected with Colonies of *E. coli*	Per Cent* of Renal Cells Infected with a Few Dispersed Bacteria
100	3 0	10
50	4 0	13
0	3 5	6

* Two hundred kidney cells were counted for each concentration of urea

only 3 to 4 per cent of kidney cells contained colonies of bacteria, and the concentration of urea did not influence their growth

Effect of Proteus *Urease Activity on* pH in Vivo *and* in Vitro

Because it was postulated that selective renal injury from *Proteus* infection was related to the alkalinity resulting from the decomposition of the high concentrations of urea peculiar to that organ, a study was made of the pH changes that accompanied growth of *Proteus, in vivo* and *in vitro.*

(*a*) *Comparison of* pH *at renal concentrations of urea with that at extrarenal concentrations during growth of* Proteus in vitro. Six strains of *Proteus* were inoculated into a liquid medium containing serial twofold dilutions of urea in concentrations ranging from 1.0 to .015 per cent. The medium was composed of 2 per cent peptone and basic salts After 18 hours the pH, as measured with a Beckman pH meter, was found to vary with each concentration of urea, as shown in Table IV.

The pH values at each concentration of urea were strikingly uniform for each bacterial strain and a marked difference was noted between renal

TABLE IV. EFFECT OF UREA CONCENTRATION ON pH DURING GROWTH OF *Proteus in Vitro*

	Concentrations of Urea (milligrams per cent)							
	1000	500	250	125	62	31	15	0
			Renal				Extra-renal	
	pH values after 18 hours incubation							
P. morgani (Smith)	9.15	8.89	8.77	8.32	7.88	7.47	7.37	7.38
P. rettgeri (Stein)	9.18	9.10	8.72	8.31	7.80	7.17	7.28	7.27
P. rettgeri (Purbaugh)	9.13			8.38			7.38	7.28
P. morgani (Owens)	9.11			8.32			7.45	7.54
P. mirabilis (North)	9.12	9.00	8.71	8.32	7.87	7.50	7.33	7.29
P. morgani (Biryle)	9.09	8.99	8.72	8.25	7.71	7.43	7.25	7.28

and extrarenal concentrations. At 15 mg. per cent, a concentration found normally in body fluids outside the kidney, the pH did not rise above the normal physiologic levels. At the high concentrations of 125 to 250 mg. per cent found in the kidney, the pH rose markedly to values ranging from 8.25 to 8.77.

(*b*) *Comparison of urinary* pH *of* Proteus *pyelonephritis with that of* Escherichia coli *pyelonephritis.* Urines were aspirated with a syringe and needle from the bladders of anesthetized pyelonephritic rats 1 week after infection, and the pH of urines was measured in a Beckman pH meter with the results shown in Table V.

TABLE V. URINE pH OF PYELONEPHRITIC RATS

Etiology of Pyelonephritis	Total Number of Rats	pH Range	pH Mean
Proteus mirabilis	12	7.70–8.52	8.14
Escherichia coli	12	6.53–7.90	7.24

The difference in the two groups is statistically significant (P = < .01).

(*c*) *The* pH *of pyelonephritic lesions during preinflammatory stage.* The pH of pyelonephritic lesions, occurring 18 hours after inoculation of *P. mirabilis*, was determined by applying the glass electrode of the Beckman portable pH meter to the cut surface of the kidney at the site of the lesion. It was then compared with the pH of nondiseased portions of the same kidney. The condition of the lesions at 18 hours is shown microscopically in Figure 2a, it is characterized mainly by selective bacterial localization in tubular epithelium with tubular necrosis and only minimal inflammatory reaction. Nine rats were infected and the lesions in 3 were large enough at 18 hours to provide a surface that

TABLE VII Relationship of Urea Concentration to pH and Kidney Cell Damage After Inoculation of *Proteus mirabilis* into Tissue Culture

Hours After Infection	Urea Concentration (milligrams per cent)	pH	Per Cent* of Damaged Kidney Cells
0	6	7.70	2
0	100	7.76	1.5
0	200	7.74	2
0	300	7.76	2
4	6	7 01	4
4	100	7.67	2 5
4	200	8 30	1 0
4	300	8 45	2 5
8	6	6 23	1
8	100	7.71	9
8	200	8 37	20
8	300	8 64	17.5
24	6	6 47	12
24	100	8 23	88
24	200	8 69	100
24	300	8 89	100

* Innumerable intracellular and extracellular bacteria were present at 24 hours in all concentrations of urea

per cent the cells had lost their cell wall, the nuclei had suffered extreme pyknosis, and the cells retained the stain poorly In concentrations of 200 mg per cent at 24 hours, the cell walls were present but disrupted, the nuclear chromatin had lost its fine reticular pattern, the nuclear outlines were irregular, and cytoplasmic vacuolization was severe. The cells still showed fairly good differential staining properties.

At concentrations of 200 and 300 mg. per cent urea all cells had been torn away from the wall of the test tube, at 100 mg. per cent urea a few normal cells were still adherent to the glass wall, and at 6 mg. per cent urea the epithelial layer appeared undisturbed on the wall of the test tube

Intracellular growth of *P. mirabilis* in the presence of extracellular streptomycin (50 micrograms per milliliter) produced at 24 hours the changes shown in Table VIII.

These findings demonstrate that intracellular growth by itself does not produce apparent injury unless high alkalinity develops.

Table IX demonstrates that *E coli*, unlike *Proteus*, produced acidity and almost no cell damage despite heavy growth when inoculated into tissue cultures with concentrations of urea up to 300 mg. per cent

TABLE VIII Relationship of Urea Concentration to pH and Kidney Cell Damage After Inoculation of *Proteus mirabilis* into Tissue Culture in the Presence of Extracellular Streptomycin

Concentration of Urea (milligrams per cent)	pH	Per Cent of Cells Damaged
6	7 83	0
100	7 88	0
200	8 0	2
300	8 20	7

TABLE IX Production of Acidity Without Kidney Cell Damage by Heavy Growth of *Escherichia coli* in Tissue Cultures Containing Varying Concentrations of Urea

Hours After Infection	Urea Concentration (milligrams per cent)	pH	Per Cent* of Kidney Cells Damaged
0	6	7 75	1
0	100	7 83	1
0	200	7 82	2
0	300	7 92	2
4	6	6 81	2 5
4	100	6.80	1
4	200	6 73	1
4	300	6 81	3
8	6	6 20	1
8	100	6 08	1
8	200	6 12	2 5
8	300	6 09	1
24	6	6 90	9
24	100	6 75	5
24	200	6 93	6
24	300	6 78	9

* Innumerable extracellular bacteria were present at 24 hours in all concentrations of urea

(d) Injury of renal epithelium in tissue culture by alkalinity created by Proteus *urease of dead bacteria, and by Jack bean urease.* The following experiment was performed in order to demonstrate that urease alone, in the absence of viable bacteria, could injure kidney epithelium. Jack bean urease, in a concentration of 10 mg per milliliter, was Seitz-filtered for sterilization and inoculated into tissue cultures of monkey kidney epithelium Another set of tissue cultures of monkey kidney epithelium were inoculated with 0 5 ml. of a suspension of acetone-killed *P mirabilis*

GENERAL DISCUSSION

DR. FREEDMAN: I should like to make one comment with regard to the specificity of *Proteus* in forming stones in experimental animals.

We have encountered stone formation in the rabbit under a variety of different circumstances These stones have formed about six to eight weeks after the injection of 10,000 staphylococci into the medulla of the rabbit kidney. This produces a local lesion.

The same type of stone formation is seen after multiple injections of *Escherichia coli* into the rabbit kidney. It is necessary to inject the kidney a number of times with *E. coli*, in contrast to the staphylococcus, which must be injected but once

Stones have been encountered in about one-third of our animals in which no bacteria were introduced into the kidney at all, and in which we produced microburns in the renal papilla. Cultures of these kidneys were sterile. The common denominator for stone formation in these experiments would seem to be not bacterial infections, but rather renal papillary injury.

DR. RELMAN: I should like to go back to what I consider to be an extremely pertinent observation made by Dr Kass in the discussion earlier this morning.

Dr. Kass pointed out that one needs constantly to bear in mind the problem of the relationship, if any, between an experimental model and the clinical phenomenon of chronic pyelonephritis. I think the model which Dr. Vivaldi and Dr. Kass and their associates have shown us this morning goes a long way toward reproducing the clinical disease, but I wish to point out that there are still a few problems with which we need to be concerned. We should be very much happier, I think, if we could have an experimental model which would show progression of renal disease and development of hypertension in the absence of sustained bacteriuria and in the absence of any evidence of active infection in renal tissue. In my own experience and, I suspect, in the experience of most other clinicians who see much of this disease, chronic pyelonephritis is often found without significant bacteriuria and may progress over a period of years in the absence of any clinical evidence of active bacterial infection.

without an increase of pressure in the bladder we can really have a movement of microbes in the ureter upstream against a freely flowing urine and against the peristalsis of the ureter.

My associate, Dr. Prát, and his co-workers have been doing some experiments with hematogenous infection in which they have been producing a partial stenosis of the ureter in rabbits, and during this partial stenosis they have been injecting *Escherichia coli*. Pyelitis was produced in 64 per cent of these rabbits, whereas the kidney was practically not affected, with the exception of two in which the infection has been very heavy.

If they kept these rabbits four to six months longer they obtained in 100 per cent of the rabbits changes which were obviously chronic pyelonephritis, which was fanning out from the pelvis.

These findings indicate that it was a hematogenous infection. When there is a slight or partial interference with the free flow of urine, the infection penetrates into the renal pelvis through the kidney and produces the process in the mucous membrane of the pelvis, and from there the process spreads into the kidney secondarily.

Dr. Prát has some experiments on complete obstruction of ureters which lasted for 36 hours He injected *E coli*, and an acute pyelonephritis was produced in 100 per cent of the animals If he left these animals alive and examined their kidneys some five to six months later, he found chronic contracted pyelonephritic kidneys with sterile urine This is the condition which Dr Relman has just mentioned.

DR DAVIS I should like to ask Dr Braude if he has made any notations of damage done by infections with staphylococci similar to that produced by the *Proteus* Some years ago we studied a large number of strains of staphylococci and found that a small proportion of them produced substantial quantities of urease This study, which was published in Young's *Practice of Urology* in 1926, was brought about by the observation that some patients with staphylococcic infections apparently had unusual quantities of ammonia in the voided urine

DR. BRAUDE All of the strains of staphylococci that we have examined have also been found to have active urease.

Because we find a difference between the localization of the organisms in *Proteus* and staphylococcal infections in the kidney, we have come to feel that there are other factors besides urease that are important in the development of this phenomenon For example, we have tested a variety of nonpathogenic saprophytic organisms that are known to have high concentrations of urease, such as *Bacillus pasteurii*. This totally nonpathogenic organism was unable to establish itself in the kidney under any circumstances.

I think that one of the crucial factors in this phenomenon of intracellular parasitism as it relates to urease concerns whether or not there are other fundamental conditions present that allow the organism to penetrate the cell. Shepard, who has studied the penetration of epithelial cells (he worked mainly with HeLa cells in tissue culture), has demonstrated that there are a variety of factors that influence different bacterial species with respect to their ability to gain entrance into epithelial cells and to multiply there.

I should like to emphasize that while we believe that urease is one of the important factors determining whether or not *Proteus* proliferates in the cell, it is by no means the only one, and these other factors may be responsible for the difference between the staphylococcus which has urease and the *Proteus*.

I also should like to mention that the observation Dr. Freedman made, that staphylococci produced stones in rabbits, is not at all surprising. Stones have been shown to be associated with staphylococcal infections in the kidney in the past, presumably because of the alkalinity produced by their urease In addition, as he indicated, there are a number of other things that can cause the formation of stones during renal injury, and infection is by no means the only one

One of the important points to be made in this connection is that the stones that form in connection with urease activity are $MgNH_4PO_4$ stones I should like to know whether he analyzed the stones that he found and whether they have this composition.

There are other factors, of course, that would have to be taken into account, such as the diet of the animals, the natural alkalinity or acidity of the species of rabbits he was dealing with, and so on We selected a group of rats that did not develop stones to any appreciable extent spontaneously, nor with any other bacterial infection

DR. DOOLAN I thought that anatomically the lymphatics to the ureter were distributed segmentally Just for my own information, I wonder if Dr. Murphy would clarify the route that bacteria might take.

Also, I thought some of the renal lymphatics drained from the cortex peripherally

DR MURPHY The studies which were made by Parker on human beings consisted of injecting some sort of injection mass in the fetus or young infants. She demonstrated pathways from the bladder to the lower ureter, then lost them in the mid-ureter and could not find them She thought they went into the wall, but was not certain Then she demonstrated communications between the upper ureter and the trunks draining the parenchyma.

As far as I know, there has been no demonstration that the renal lymphatics drain peripherally; that is, all of the demonstrated work, including Rawson's, shows that everything drains centripetally toward the hilus and into the major lymphatic trunks around the vessels.

DR BEESON· Dr Murphy, the deposit you found in the medulla — isn't it just as possible that this was some kind of pyelolymphatic backflow? You must have postulated an immense lymphatic flow from the ureter up across the pelvis and into the tip of the papilla, in order to get a deposition like that

DR. MURPHY I am not certain how the deposit gets there, but there was none in the collecting system. Only one dog in twenty-five studies refluxed, and this with great pressure Although there was dye in the pelvis in this animal, it did not stain the renal pyramid.

DR. BEESON You don't think it could be washed back down in other cases, or didn't you see the dye?

DR MURPHY It could be, but we usually clamp the ureters at the bottom and open them up from the top down

DR. CROSLEY I should like to comment on the localization of infection in the medulla In recent years it has been observed by a number of workers that there is an increased osmolality of the renal tissues starting with the outer medulla and extending toward the papilla This raises the question whether anyone has studied bacterial growth in the presence of increased osmolality.

Secondly, in the animals studied, is there any evidence that the earliest lesions occur in the tips of the papillae, in contrast to starting in the outer medulla?

DR JACKSON A few years ago we did the experiment to which you have referred, taking urine from both normal patients and those with pyelonephritis, filtering it to make it cell-free and bacteria-free, and then altering its composition by the introduction of various components, albumin, globulins, urea, and a variety of salts and sugars.

Under these circumstances there was no significant influence of the osmolality or of any of these factors that we could demonstrate. Urea caused no increased growth, pH, of course, had a significant effect, and glucose increased the rate of proliferation of bacteria but affected the total growth relatively little.

to have a higher local pH with cell injury than we could ascertain from the mean determination However, we also fail to observe at this point any discernible cell injury, and if it occurred it would have to be of a type that could be determined morphologically.

DR. EPSTEIN· As Dr. Crosley pointed out, in the normal kidney the concentration of urea is much higher at the tip of the papilla than it is at the cortex Has Dr. Braude observed in his *Proteus* infections that the amount of histological infection was greater in the papilla, where the concentration of urea is elevated, than it was in the cortex?

DR. BRAUDE. Actually, the reverse is true, Dr. Epstein. As you could see from the slide, the heaviest localization of organisms was in the cortex and outer medulla

We have additional data, however, which time did not allow me to present, that will help to answer this. There is an optimal concentration of urea with respect to penetration of the organisms into the tubular epithelial cells, and this is 200 mg. per cent. As one goes up to 300 mg. per cent, even though greater alkalinity may result, the number of organisms entering the cell is reduced

This is one of the reasons why I hesitate to accept Dr. Rowley's suggestion that injury to the cell may be responsible for penetration, because even though at the higher concentrations of urea there are higher alkalinity, greater injury, and earlier injury, there is less penetration of organisms into the cell.

We have found that the tissue cells have to be in tiptop shape (as Shepard has pointed out) for any organism to invade them

DR. NETER In view of the problem raised concerning the possible development of chronic pyelonephritis from acute bacterial pyelonephritis, and the conceivable pathogenetic role of bacterial urease and endotoxin, are there any experiments to indicate the duration of the persistence in the kidney of these materials? This question could be approached either by the Coons fluorescent antibody technique or, as Dr. Rowley has done, by growing radioactively labeled *Proteus* and determining the persistence of radioactivity in the kidney.

DR. SANFORD. We have carried out studies along these lines by producin pyelonephritis in rats according to the technique described by Braud The course of infection with immunologically specific strain of *Escher chia coli* (O-III B-4) has been followed by "staining" sections of kidn with a fluorescent labeled antiserum against the O-III antigen As the ac infection subsides, the residual scars show an extensive mononuclear

ction; however, the kidneys are sterile by the usual bacteriological tech-
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ntigen no longer can be detected.

SUMMATION

PAUL B. BEESON, M.D.
(New Haven, Connecticut)

It is now my task to attempt a summation of this morning's program. We have heard a good deal of discussion of pathways of infection, which is a matter of very great practical importance. If one believes that most cases of bacterial infection of the kidney result from hematogenous dissemination, he does not worry very much about the induction of infection of the bladder urine.

This has certainly been the philosophy of many people who have been inclined to accept the idea that most cases of infection of the kidney do result from infection brought by way of the blood stream. As Dr Brod pointed out, the "ascending" pathway is opposed by ureteral peristalsis and the direction of the urine flow. It has been difficult, as Dr Murphy indicated, to show a direct lymphatic connection between the bladder and the kidney In spite of the objections, I favor the theory of "ascending" infection in the pathogenesis of pyelonephritis I don't worry so much about the calculations of the rate of upward diffusion that Dr. Kass mentioned It seems to me that peristalsis is not always going on in the ureter, that there are times when the ureter is a limp tube, and that any increase in pressure from below will send fluid shooting all the way to the top of the ureter.

With recent development of techniques of the delayed cystogram and, even more important, the use of postmicturition cystograms and the technique of cinefluorography, it has been possible to show that this kind of rapid movement of fluid from the bladder all the way up to the kidney is not an uncommon thing, and does not always require gross disease of the lower urinary tract for its occurrence. I would suggest that this is probably the simple mechanism of transport of bacteria in fluid from the bladder cavity to the kidney.

Dr. Gorrill noted that most people who have worked in the field of experimental pyelonephritis have preferred the hematogenous method of infecting the kidney. It is simpler, you can be sure that the dose which reaches the kidney is unvaried, and you can be sure of the time at which

to have a higher local pH with cell injury than we could ascertain from the mean determination. However, we also fail to observe at this point any discernible cell injury, and if it occurred it would have to be of a type that could be determined morphologically.

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to have a higher local pH with cell injury than we could ascertain from the mean determination However, we also fail to observe at this point any discernible cell injury, and if it occurred it would have to be of a type that could be determined morphologically.

DR. EPSTEIN: As Dr. Crosley pointed out, in the normal kidney the concentration of urea is much higher at the tip of the papilla than it is at the cortex. Has Dr. Braude observed in his *Proteus* infections that the amount of histological infection was greater in the papilla, where the concentration of urea is elevated, than it was in the cortex?

DR BRAUDE Actually, the reverse is true, Dr. Epstein. As you could see from the slide, the heaviest localization of organisms was in the cortex and outer medulla.

We have additional data, however, which time did not allow me to present, that will help to answer this. There is an optimal concentration of urea with respect to penetration of the organisms into the tubular epithelial cells, and this is 200 mg. per cent. As one goes up to 300 mg per cent, even though greater alkalinity may result, the number of organisms entering the cell is reduced

This is one of the reasons why I hesitate to accept Dr. Rowley's suggestion that injury to the cell may be responsible for penetration, because even though at the higher concentrations of urea there are higher alkalinity, greater injury, and earlier injury, there is less penetration of organisms into the cell.

We have found that the tissue cells have to be in tiptop shape (as Shepard has pointed out) for any organism to invade them

DR. NETER: In view of the problem raised concerning the possible development of chronic pyelonephritis from acute bacterial pyelonephritis, and the conceivable pathogenetic role of bacterial urease and endotoxin, are there any experiments to indicate the duration of the persistence in the kidney of these materials? This question could be approached either by the Coons fluorescent antibody technique or, as Dr. Rowley has done, by growing radioactively labeled *Proteus* and determining the persistence of radioactivity in the kidney.

DR. SANFORD We have carried out studies along these lines by producing pyelonephritis in rats according to the technique described by Braude. The course of infection with immunologically specific strain of *Escherichia coli* (O-III:B-4) has been followed by "staining" sections of kidney with a fluorescent labeled antiserum against the O-III antigen As the acute infection subsides, the residual scars show an extensive mononuclear re-

the kidney, so to speak, is enough to light up an infection and cause a massive suppurative process there. It is hard to see how growth of bacteria in the stagnant urine can be the key to this process.

It also has interested us that the most susceptible preparation is a relatively normal kidney which has only recently been subjected to some obstructive lesion. The longer the obstructive lesion persists, the less susceptible the preparation is Dr Guze, when he was at Yale, developed a method of causing partial occlusion of the ureter by means of a radiation injury. This did produce a striking hydronephrotic lesion, and yet there was surprisingly little increase in susceptibility to infection in this kidney with an enormously distended pelvis (and undoubtedly with stagnation of urine flow).

Well, what could the effect of obstruction be? Obviously, when there is increased hydrostatic pressure in the urinary passages there must be alterations in the flow of blood through the organ, and there must also be alterations in lymphatic circulation, and it may be that one or the other of these is responsible Dr. Gorrill suggested this morning that the collection of edema fluid may be the key I think this is an interesting suggestion, but it is hard for me to see just how this would act The edema fluid would presumably contain the humoral substances that are part of our normal defense mechanisms. Phagocytosis ought to be operating normally, and although no one would deny the fact that there is edema fluid, its role in the great susceptibility that goes with obstructive uropathy is a point that needs to be demonstrated

Dr Zollinger's report on phenacetin and increased susceptibility of the kidney is certainly fascinating, and one wonders why, if the consumption of phenacetin in this country is as great as it is in his country, our clinicians and pathologists have not recognized the relationship As far as I know, it has not appeared to be a factor in kidney infections in this country, but we must obviously be on the alert for it. Whether the lesion that he and other European pathologists have noted is due to chemical injury of the kidney or is the result of chemical injury plus increased susceptibility to infection remains to be determined

Dr. Relman and then Dr. Brod raised a point which I am sure is going to come up over and over again in the next two and a half days, and that is whether all this talk about infection has anything really to do with *chronic* pyelonephritis, the disease we are all interested in trying to prevent This is a very good point, and I must say that there are some impressive reasons for arguing that the entity known as acute pyelonephritis may not be the one that is a forerunner of the disease that w chronic pyelonephritis One of these points is the far greater i of acute pyelonephritis in females There is no such prepon chronic pyelonephritis in the female.

cal injury of the papilla followed by introduction of bacteria, these wedges of infection surrounded by normal, uninfected tissue are the usual result. We have been fascinated by watching these, noting that the infection never seems to spread into the normal tissue adjacent, even though the pelvic urine is loaded with pus and bacteria. We are also struck by the fact that the infection tends to die out in a period of weeks even within these wedges in infections caused by coliform bacteria

I think it is certainly possible to conceive of this being the sort of lesion that may develop in the human being over a long period of time, and that chronic pyelonephritis may be the result of a series of minute little wedges of infection occurring over periods of years, none of them being of such size as to produce clinical manifestations.

Of course the possible reservoir for continuing infection between the episodes of pyelonephritis (as Dr. Kass' work has so clearly shown) may be that the bacteria continue to grow in the urine, then reinfect the kidney from time to time. It may be that various types of lesions, particularly in the medulla, such as the deposition of calcium, a small vascular lesion, an area of scarring due to some previous active infection, may set up a condition of susceptibility within a certain group of nephrons, and then the bacteria in the urine bathing this area may be able to produce another focus of infection.

One of the subjects that have received very little attention this morning has been the role of obstruction. Of course this is the one thing that we can be sure of as tending to cause infection in the urinary tract.

In this regard it seems unfortunate that a misleading term has been introduced into medical texts, namely, "stagnation of urine." I believe this term has caused stagnation of thinking about the whole problem. The word "stagnation" seems to imply that if the urine sets around in the passages long enough, bacteria will have a greater opportunity to grow, and that the number of organisms present in the urinary tract will thereby be larger A number of objections can be raised to this as being the real mechanism responsible for the effect of obstruction on the susceptibility of the kidney. One thing that has been shown in experimental work is that the period of occlusion of the ureter can be very brief (in some of Brainerd's work the ureter was kinked for only fifteen minutes) and yet a massive pyelonephritis can ensue following the introduction of bacteria in such preparations. It is hard to see, then, how stagnation of urine flow could have been responsible

In some of the experiments in our laboratory, rats were injected intravenously with *Escherichia coli*, then allowed to rest for three or more days until the period of bacteremia had subsided and until there were only a few organisms persisting in the kidneys, and then the ureter was tied As I have said before, there is no excretion of these bacteria into the urine and yet this occlusion of the ureter, when bacteria are resting in

the structural-functional relationships which integrate the individual units into a functional whole. The events which occur in a single limb of a loop of Henle, or in a single collecting duct or blood capillary, are determined by the activity of adjacent units in the particular region of the medulla.

The current concept, as developed by Wirz[81, 82] and Gottschalk and Mylle,[23] places the primary event of the concentrating mechanisms in the ascending limbs of the loops of Henle. (In the inner zone of the rat medulla these are exclusively thin limbs, in the inner stripe of the outer zone, some are thin but most are thick limbs.) It is proposed that as a consequence of the reabsorption of sodium chloride by the relatively water-impermeable cells of the ascending limbs, water moves passively from the freely permeable descending limbs and collecting ducts In this view, the ascending limbs are the "creative" elements in a countercurrent multiplier system. There is also a countercurrent exchange mechanism which helps preserve the differences in osmolality created by the multiplier system· the blood capillaries of the medulla

In the outer zone of the medulla (inner stripe) the cell morphologist encounters relatively little difficulty in finding structural counterparts of the proposed functions He can readily ascribe an active role to the thick limbs of Henle's loops Its cells are more tightly packed with mitochondria (Figure 20), its mitochondria with more "cristae" (Figure 21), than any other cell in the kidney (Figure 22), its level of staining for DPNH-tetrazolium reductase activity is the highest of any renal cell (Figure 18, also see reference 75) There is a high level of ATPase activity in the cell membranes covering the extensive interdigitations with adjacent cells, its reaction product is shown in Figure 19, as viewed in the light microscope (see reference 68 for other light micrographs and reference 49 for electron micrographs) In contrast, the cells in the thin descending portion of Henle's loop seem ideally suited to the relatively passive role assigned them They have few mitochondria and these are small (Figures 21 and 22) They show little DPNH-tetrazolium reductase activity (Figure 18), and there is no ATPase activity demonstrable in their membranes (Figure 19) There are numerous "conduits" crossing the cells (Figures 21 and 22) – or between adjacent cells[65] – which may facilitate movement of water. The collecting duct cells show, at their bases, many infoldings of the cell membranes and, between adjacent cells, numerous interdigitations (Figure 20), to the morphologist these, too, appear to be devices which may aid water movement Finally, the blood flows through this region of the medulla in afferent and efferent capillaries arranged parallel to each other and grouped together to form the vascular bundles or vasa recta Each capillary tends to be surrounded by several capillaries of the other type, carrying blood in the other direction

lished acid phosphatase method of Barka[a] and the benzidine reaction The acid phosphatase reaction product is red and can be distinguished readily from the brown peroxidase reaction product. Whereas 30 minutes after injection brown luminal pinocytosis vacuoles are seen separated from the more basal red-stained lysosomes, 90 minutes later there are only mixed red-brown lysosomes.

Preliminary observations have been made of cells in the proximal convolutions of animals killed 18 hours following intraperitoneal injection of the same low dose of peroxidase as was used for the intravenous injections. Perhaps because of a slower filtration rate in the kidney, a more diffuse reaction was found similar to that described by Oliver *et al.*[53] after intraperitoneal injection of hemoglobin. The manner of entry into a cell and its disposition there may vary with the nature and amount of the protein.

Since there is no sensitive method for following the "endogenous" proteins reabsorbed by the cell, we cannot suggest how they enter the cell. Presumably, in the cells with large lysosomes (in most proximal convolutions of mature male rats and in few convolutions of female rats) protein is constantly being reabsorbed in some fashion and segregated in the lysosomes — perhaps in the Golgi apparatus (Figure 5).

It may be assumed that within the lysosomes the protein is digested by the action of cathepsins that are localized there. The primary significance of Oliver's studies of the protein absorption droplets of pathological conditions, in experimental animals and in human disease, is "that the formation of the droplets is related to and hence can be considered an *abnormal modification of a process that is carried on by the proximal* convolutions of the nephrons, namely, the reabsorption and disposal of plasma proteins." [54] Our work gives support to this important concept. In our conclusions the role of the mitochondria is de-emphasized[b] and the major role in protein segregation and digestion is assigned to the lysosomes.

The Loops of Henle and the Collecting Ducts

Of the many remarkable features of the mechanisms by which urine is concentrated in the medulla, none is more impressive to the biologist than

[a] We wish to thank Dr T. Barka of the Mount Sinai Hospital, New York, for providing us with the details of this method

[b] We will describe elsewhere the mitochondrial changes which we have seen in cells with protein absorption droplets, and we will consider those described by Rhodin[62] and others (e g., Miller and Sitte[38]) in protein-injected mice We believe that these changes occur *after* the formation of protein absorption droplets, in cells which have absorbed much protein. Miller,[38] in a review seen when our studies had been completed, has also concluded that the mitochondrial changes are not primary in the formation of protein absorption droplets

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s
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to DPNH-tetrazolium reductase activities, that TPN-dependent sequences may be of special significance in the metabolism of these cells.

The *collecting duct cells* in the papilla, as in the outer zone of the medulla, have infoldings at their bases and lateral interdigitations between adjacent cells. Perhaps these are related to the duct's permeability to water and substances like urea.[4] There is some suggestion that these differentiations of the cell surface may vary in the apical and basal halves of the papilla (Figures 25 and 26), and with the extent of dehydration of the medulla (Figure 26), but this requires further study. ATPase activity is demonstrable in the infolded and interdigitated areas of the cell membranes in the apical half (Figure 28) but not in the basal half of the papilla; on the other hand, the blood capillaries normally show intense ATPase activity only in the basal half (Figure 19). In the basal half of the papilla, there are still "intercalated" or "dark" cells which, as in the cortex,[5, 43] show numerous luminal microvilli and many large vacuoles. The membranes of the vacuoles near the cell surface not uncommonly are continuous with the cell membranes. These may, then, be pinocytosis vacuoles forming, or they may be vesicles opening their contents to the surface. The latter possibility is of interest in relation to the suggestion of Yoshimura and Nemoto[43] that they produce an apocrine secretion, and to Ginetzinsky's suggestion[16] that hyaluronidase is secreted by collecting duct cells in apocrine gland fashion. Consistent with their large number of mitochondria, the "dark" cells stain intensely with the DPNH-tetrazolium technique (Figure 18) or in Baker's acid hematin preparation.[1] It is interesting that TPNH-tetrazolium reductase activity, apparently localized in the mitochondria in the cells of the proximal and distal convolu-

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FIGURE 25. [illegible] ×12,400. The [illegible] (cd), marked [illegible] vesicles (pino- [illegible] swelling may [illegible] er content of [illegible] interstitial cells (i) show numerous dark lipid droplets, ergastoplasm, and sharply defined cell membrane; the cells are partially surrounded by fibrous material resembling the basement membrane.

FIGURE 26. Cells in the papilla (apical half) of a hydrated rat. ×8800. The plane of section of the collecting duct (cd) is not through the basal infoldings of the cell membranes. Between adjacent cells there are widened spaces (arrows) probably due, if not to inadequate fixation, to increased water content of the collecting duct. Note that the interstitial fibers (f) resemble the basement membranes of the capillary (V) and collecting duct, with which they are continuous, and that the fibers partially enclose the *interstitial cells* (i). The interstitial cells make more intimate contact with the capillary than collecting duct; they contain numerous dark lipid droplets.

v
v
b
c
b
v
v
27



tions,[19] is not correlated with mitochondrial number in collecting duct cells. The "dark" cells are no more intensely stained than the "light" cells. The duct cells in the apical half of the papilla, where they lack "dark" cells, stain more intensely than in the basal half of the papilla. We have already noted that the cells in the thin limbs of Henle's loop, although possessing few mitochondria, stain relatively intensely for TPNH-tetrazolium reductase activity.

The *blood capillaries* of the papilla appear to be of one type. Nearer the outer zone, one may find in the inner zone of the medulla vessels like the afferent capillaries of the vascular bundles, with relatively tall endothelial cells in which pinocytosis (?) vacuoles are numerous (Figure 27). Within the papilla we have not been able to find such vessels. All now resemble the efferent vessels of the vascular bundles in possessing endothelial cells with much attenuated cytoplasm interrupted by numerous "fenestrations" or "pores" (Figure 25) These are probably regions in which diffusion is facilitated, although it may prove that there are in fact no gaps in the cytoplasmic membrane.[b] In two respects the capillaries in the papilla resemble the afferent vessels of the vascular bundles. the presence of small microvilli at the luminal surface and long foot-like extensions (Figure 25) Occasionally, small pinocytotic-like vacuoles are seen at both luminal and basal surfaces of the cells (Figure 25).

Intimately related to the capillaries of the papilla are specialized *interstitial cells*. The abundance of these cells is a striking feature of the rat papilla. They were first observed by Vimtrup and Schmidt-Nielsen,[74] who considered them "sparse" in the rat but numerous in a certain region

[b] Fawcett and Wittenberg[13] report that in similar cells of the swim bladder of the toadfish the two cell membranes are fused into single membranes continuous over the "pores" Longley *et al*[35] reported similar findings in the efferent vessels of the vascular bundles in the outer zone of the renal medulla We find many such single membranes, but we also find quite a few discontinuities even in areas where artefact cannot readily be invoked These discontinuities may be compared with the pores described by Pease[59, 61] in the peritubular capillaries of the cortex.

FIGURE 27 Inner medulla of an untreated rat. ×15,100. The blood capillaries are indicated by v, the one to the right is sectioned roughly parallel to the base, with a nucleus of an endothelial cell seen at n In the capillary to the left, note the small pinocytosis (?) vacuoles (arrows); the darkening luminal surface is due to the phosphotungstic acid treatment,[79] which also increases the contrast of the interstitial fibrils Note that the basement membrane (b) has less electron opacity In the interstitial cell (i) numerous droplets may be seen, fibrous or granular material inside or near the ergastoplasm is shown by arrows.

FIGURE 28 Collecting duct in the papilla (apical half) of an untreated rat, processed for ATPase activity by the method of Kaplan and Novikoff.[29] ×10,000 Marked accumulation of reaction product (lead phosphate) is seen in the cell membranes where they are infolded at the base and where they interdigitate with adjacent cells (arrows). The structure below is either a capillary or thin limb of Henle's loop.


28

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of the papilla in the kangaroo rat. These authors wrote: "Perhaps they are some kind of muscle cells on small vessels, but it is difficult to exclude the idea that they have a special relation to the collecting ducts." Sternberg *et al.*,[69] using the tetrazolium staining procedure on frozen sections, found that these cells were numerous throughout the rat papilla, arranged at right angles to the collecting ducts. Noting a certain cytological resemblance to the pericapillary Rouget cells and suggesting that they may be contractile, as has been thought by some of Rouget cells, Sternberg *et al.* nevertheless considered the interstitial cells to be related to the collecting ducts rather than blood capillaries. They suggested that they might be able to constrict the lumens of the collecting ducts and thus to facilitate tubular reabsorption, and that they may be the end-organs for ADH action.

From our electron microscope as well as cytochemical studies, we can confirm the observations of Sternberg and co-workers that the interstitial cells are abundant throughout the rat papilla, and are more numerous in the basal than apical half. However, we find the cells to be closely related to the blood capillaries. The capillaries are so thin that even in 2-micron sections of good cytological quality, they may readily be overlooked in light microscopy (Figure 24). In electron micrographs we have frequently encountered intimate relations, including attachment, of interstitial cells and blood capillaries. On the other hand, although they may lie very close to collecting ducts, and more frequently to loops of Henle, actual contact seems restricted to a few fibers resembling the basement membranes (Figures 25 and 26).

If indeed the interstitial cells are contractile, this may constitute a means of regulating the blood flow. It may be related to the finding of Lilienfield *et al.*[32] that the papilla of kidneys removed from the animal (dog) and frozen is "extraordinarily deficient" in erythrocytes. It should be noted that Longley *et al.*[35] have seen these interstitial cells in close contact with the blood capillaries of the vascular bundles.

The interstitial cells may, however, have other significant functions. The ergastoplasm, which is relatively well developed in many of these cells, may show expanded areas filled with a material of moderate electron opacity. This may be a material (lipomucoprotein?) to be secreted by the cells into the interstitium and basement membranes. With phosphotungstic acid treatment of the section[79] the inner content of the ergastoplasm, and of adjacent regions, resembles more the granular and fibrous material in the interstitium.

An even more speculative role may be suggested for the striking number of large lipid droplets[1] within the interstitial cells (Figures 24–26).

[1] These have the usual appearance of lipid droplets in electron micrographs of thin sections of osmium-fixed tissue. They also stain with oil red O[22] in frozen sections. They are stained by toluidine blue in 2 μ sections of methacrylate-embedded osmium-fixed tissue (Figure 24).

May it be more than coincidence that lipid-laden cells are present in a region where cells (in the loops of Henle and collecting ducts) are present in which TPN-dependent metabolic sequences may be particularly important (see above), and that TPNH has a significant role in lipogenesis[2,50]

Another striking feature of the rat papilla is the *metachromasia* of the interstitial substance We found this metachromasia before we learned of the work of Ginetzinsky and colleagues[18,20] that ADH causes the release of hyaluronidase from the cytoplasm of the collecting duct cells, resulting in depolymerization of the metachromatic material in the basement membranes and intercellular substance of the collecting ducts Thus water may move more freely through the intercellular spaces into the hypertonic interstitium. Thus far, we have been unable to observe the metachromatic material between adjacent cells, in either light microscopic preparations (other methods of preparation might reveal it‡) or in electron micrographs (generally mucopolysaccharides are not visualized in the usual preparations of osmium-fixed tissues) We saw no changes in metachromasia of the interstitium in the single rat injected with ADH, but we did see some evidence of enlarged spaces between adjacent cells of the collecting ducts in the hydrated animal (Figure 26).

It is likely that the metachromasia is due to a polyanionic material, such as hyaluronate or chrondroitin sulfate[3,65] If, because of its charge, this material affects the movement of cations, it may be of significance that the concentration of metachromatic material increases, in gradient fashion, from the base to the apex of the papilla, the site of deepest metachromasia is the area of highest urine concentration

In the electron micrographs of thin sections, most of the interstitium consists of small granules, about 100 to 150 Å in diameter These are sometimes arranged, in linear fashion, on fine fibrils Fibrous-like material, 400 to 500 Å wide and resembling the basement membranes, is scattered irregularly throughout the interstitium Upon these fibers, which prob-

* On a number of occasions, we have observed fat droplets identical with those of the interstitial cells lying apparently freely in the interstitium, in areas with well-preserved fine structure We have twice seen such free lipid droplets in contact with the base of thin limb cells

† We have studied (1) both frozen sections and paraffin sections of tissue fixed overnight in cold formol-calcium, stained with 1×10^{-3} M toluidine blue in 20% acetone, and mounted in water, (2) paraffin sections of formol-sublimate-fixed tissue stained in 3×10^{-4} M toluidine blue in McIlvaine's buffer, pH 3.0, and treated with ammonium molybdate[36], and (3) 2 μ methacrylate sections stained in 6.5×10^{-2} M toluidine blue in water and examined in cold water As the temperature of the preparation rose to that of the room, the metachromasia diminished markedly

‡ We do not know the details of the staining procedure used by Ginetzinsky and colleagues

ably interconnect with each other and ultimately reach the basement membranes, are situated the interstitial cells. Even with phosphotungstic acid treatment,[79] which greatly improves the contrast of the fibrils, no structures with the characteristic periodicity of collagen are seen (Figure 27). It would be of interest to study changes in metachromasia and fine structure of the interstitium in pathological states.

Our discussion of the medulla began by observing that the activities of the individual units are dependent upon the region of the medulla in which they are found. In the course of the discussion we have referred to a number of differences that we have found between apical and basal halves of the papilla. These may be recapitulated briefly. In the apical half, there is more metachromasia in the interstitial material, there are fewer specialized interstitial cells, the collecting duct cells stain more deeply for TPNH-tetrazolium reductase activity; the collecting ducts lack "intercalated" or "dark" cells; the basal infoldings and lateral interdigitations of the collecting duct cells show ATPase activity; the blood capillaries show little ATPase activity.

Since biochemical or functional cytology is still in its infancy, it is hardly surprising that we cannot suggest how these observations may be related to the concentrating mechanisms or other functions of the medulla. After reviewing the renal physiologist's concepts of medullary function, Lamdin[80] concludes: "It is disconcertingly all too apparent that much of present-day hypothesizing about the mechanism(s) ultimately responsible for dilution and concentration of the urine is largely conjectural, fabrication has been out of cloth generously perforated by large gaps in knowledge. The secrets of the characteristics of the tubular membranes and their metabolic activities remain largely to be discovered."

The Distal Convolution

Except for the cells in the macula densa, the distal convolution cells are indistinguishable, by our techniques, from the cells in the thick limb of Henle's loop They exhibit high levels of apparent ATPase activity in the cell membranes over the numerous projections at the base of the cell (Figure 3). *They have high levels of DPNH and* TPNH-tetrazolium reductase activities (Figure 2; also Figures 4 and 5 of reference 49), consistent with numerous mitochondria rich in cristae.

An outstanding feature of the macula densa's cytochemistry is the relatively high ratio of TPNH-tetrazolium reductase to DPNH-tetrazolium reductase activities in its mitochondria.* Indeed, it is possible, even with

* Nachlas *et al.*[42] first noted that the macula cells stained darkly in the tetrazolium technique, when phosphogluconate was used as substrate and TPN was added to

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a 2.5 X objective, to pick out the macula cells in a TPNH-tetrazolium reductase preparation. This points again to the importance of learning more about metabolic pathways which utilize TPN. It may be relevant to mention that a survey we have made of the staining of rat tissues in the tetrazolium procedures has indicated that a relatively high ratio of TPNH-tetrazolium to DPNH-tetrazolium reductase activities characterizes cells in a number of endocrine tissues (islets of Langerhans, adrenal gland, thyroid gland, and pituitary gland).

Concluding Remarks

In his brilliant Harvey Lecture of 1944, Jean Oliver[52] wrote: "The conclusion is that the structural aspect of renal activity offers as rich and limitless field to the investigator as does the functional. All that is needed are methods appropriate to the task." We have attempted to show the value for such investigations of coupling cytochemistry and electron microscopy.

Cytochemical stains frequently permit ready identification of cytological and histological structures: DPNH-tetrazolium reductase activity for mitochondria, acid phosphatase activity for lysosomes, ATPase activity for some cell membranes, alkaline phosphatase activity for the brush border, ATPase (and "ADPase") activity for arterioles and capillaries, DPNH-tetrazolium reductase activity for glomerular podocytes and the "dark" or "intercalated" cells of the collecting ducts, TPNH-tetrazolium reductase activity for the macula densa and, to a lesser extent, the thin limbs of Henle's loops, metachromasia for the interstitial material of the papilla, lipid staining for the interstitial cells in the papilla. Injected peroxidase and other enzymatic proteins may be traced inside of tissues and cells by specific cytochemical staining procedures.

Electron microscopy reveals a bewildering array of cytological structures to which the renal physiologist will ultimately attach functional significance: multipedicled podocytes in the glomerulus, interdigitations of adjacent cells by extensions containing oriented mitochondria—in the

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the medium Hess *et al*[25] found the same to be true when glucose-6-phosphate was substituted for phosphogluconate. Recently Hess and Pearse[27] have described interesting changes in staining intensity when rats are made hypertensive by partial ligation of the renal artery. These authors attribute the staining reactions to high levels of phosphogluconate and glucose-6-phosphate dehydrogenase activities. Such high levels may indeed be present in the macula cells, but, as we have noted elsewhere,[44] this conclusion is weakened by the biochemical work indicating that these two enzymes are readily soluble. Thus, *irrespective of where localized*, the dehydrogenases may diffuse from the unfixed sections used by these investigators into the medium, where TPNH would be generated. The macula cells would then stain intensely because of their high level of TPNH-tetrazolium reductase activity.

ably interconnect with each other and ultimately reach the basement membranes, are situated the interstitial cells. Even with phosphotungstic acid treatment,[79] which greatly improves the contrast of the fibrils, no structures with the characteristic periodicity of collagen are seen (Figure 27). It would be of interest to study changes in metachromasia and fine structure of the interstitium in pathological states.

Our discussion of the medulla began by observing that the activities of the individual units are dependent upon the region of the medulla in which they are found. In the course of the discussion we have referred to a number of differences that we have found between apical and basal halves of the papilla These may be recapitulated briefly. In the apical half, there is more metachromasia in the interstitial material; there are fewer specialized interstitial cells; the collecting duct cells stain more deeply for TPNH-tetrazolium reductase activity; the collecting ducts lack "intercalated" or "dark" cells; the basal infoldings and lateral interdigitations of the collecting duct cells show ATPase activity; the blood capillaries show little ATPase activity.

Since biochemical or functional cytology is still in its infancy, it is hardly surprising that we cannot suggest how these observations may be related to the concentrating mechanisms or other functions of the medulla. After reviewing the renal physiologist's concepts of medullary function, Lamdin[80] concludes: "It is disconcertingly all too apparent that much of present-day hypothesizing about the mechanism(s) ultimately responsible for dilution and concentration of the urine is largely conjectural, fabrication has been out of cloth generously perforated by large gaps in knowledge. The secrets of the characteristics of the tubular membranes and their metabolic activities remain largely to be discovered."

The Distal Convolution

Except for the cells in the macula densa, the distal convolution cells are indistinguishable, by our techniques, from the cells in the thick limb of Henle's loop. They exhibit high levels of apparent ATPase activity in the cell membranes over the numerous projections at the base of the cell (Figure 3). They have high levels of DPNH and TPNH-tetrazolium reductase activities (Figure 2, also Figures 4 and 5 of reference 49), consistent with numerous mitochondria rich in cristae

An outstanding feature of the macula densa's cytochemistry is the relatively high ratio of TPNH-tetrazolium reductase to DPNH-tetrazolium reductase activities in its mitochondria.* Indeed, it is possible, even with

* Nachlas *et al.*[12] first noted that the macula cells stained darkly in the tetrazolium technique, when phosphogluconate was used as substrate and TPN was added to

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a 25 X objective, to pick out the macula cells in a TPNH-tetrazolium reductase preparation. This points again to the importance of learning more about metabolic pathways which utilize TPN. It may be relevant to mention that a survey we have made of the staining of rat tissues in the tetrazolium procedures has indicated that a relatively high ratio of TPNH-tetrazolium to DPNH-tetrazolium reductase activities characterizes cells in a number of endocrine tissues (islets of Langerhans, adrenal gland, thyroid gland, and pituitary gland)

Concluding Remarks

In his brilliant Harvey Lecture of 1944, Jean Oliver[52] wrote "The conclusion is that the structural aspect of renal activity offers as rich and limitless field to the investigator as does the functional All that is needed are methods appropriate to the task." We have attempted to show the value for such investigations of coupling cytochemistry and electron microscopy.

Cytochemical stains frequently permit ready identification of cytological and histological structures DPNH-tetrazolium reductase activity for mitochondria, acid phosphatase activity for lysosomes, ATPase activity for some cell membranes, alkaline phosphatase activity for the brush border, ATPase (and "ADPase") activity for arterioles and capillaries, DPNH-tetrazolium reductase activity for glomerular podocytes and the "dark" or "intercalated" cells of the collecting ducts, TPNH-tetrazolium reductase activity for the macula densa and, to a lesser extent, the thin limbs of Henle's loops, metachromasia for the interstitial material of the papilla, lipid staining for the interstitial cells in the papilla. Injected peroxidase and other enzymatic proteins may be traced inside of tissues and cells by specific cytochemical staining procedures

Electron microscopy reveals a bewildering array of cytological structures to which the renal physiologist will ultimately attach functional significance multipedicled podocytes in the glomerulus, interdigitations of adjacent cells by extensions containing oriented mitochondria — in the

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TABLE III. Properties of Human Renal Phosphatases

	Alkaline Phosphatase	ATPase	G-6-Pase	Acid Phosphatase
Substrate	p-nitrophenylphosphate	ATP	G-6-P	p-nitrophenylphosphate
K_m	0 35 mM	2 5 mM	4 6 mM	4 3 mM
Optimum substrate concentration	5 mM	3 5 mM	>15 mM	>18 mM
Optimum pH	10 0	8 44	6 35	5 45
Activity vs incubation time	Linear up to 1 hr	Linear up to 75 min	Linear up to 90 min	Linear up to 60 min
Activity vs enzyme concentration	Linear over 0 7 mM/h/L (13% utilization)	Linear up to 1 65 mM/h/L (55% utilization)	Linear up to 0 41 mM/h/L (2 3% utilization)	Linear up to 0 32 mM/h/L (1 8% utilization)
v2°/v37°	0 17	0 046	0 064	0 23
Stability	24 hr at +4° C −0% 24 hr at −30° C. −0%	24 hr at +4° C. −85% 24 hr at −30° C. −63%	30 min at 37° C −65% 24 hr at −30° C. −22%	24 hr. at +4° C. −0% 24 hr at −30° C. −0%
Optimum Mg^{++} concentration	2 mM	1 mM	0 mM	3 mM
Effect of Mg^{++}	0 mM Mg^{++} −18 7% 2 − 0 0%	0 mM Mg^{++} −79% 0 5 −10% 1 − 0% 2 −13% 5 −32% 10 −44%	0 mM Mg^{++} −0 0% 2 −4 7% 5 −7,1%	0 mM Mg^{++} −18% 1 − 6% 3 − 0% 10 − 4%
Effect of 5 mM Mn^{++}	−13%	− 27%	−1 5%	+3 9%
Effect of 10 mM CN^- 10 mM I^- 2 5 mM MoO_4^{--}	−98 0% − 6 7% −13 9%	+ 6 6% + 8 3% −21 0%	0% −35% −42%	+10% −41% −64%

TABLE IV Alkaline Phosphatase Activity in the Nephron of Various Species *

	Man 7†	Monkey 2	Dog 3	Rat 2	Rabbit 2	Frog 2	Toadfish 2
Glomerulus	0.50 ± 0.69 (33)‡	0.60 ± 0.081 (18)	1.90 ± 0.15 (19)	9.7 ± 0.88 (10)	1.03 ± 0.25 (11)	29.8 ± 3.8 (13)	—
Proximal convoluted tubule	5.56 ± 0.29 (38)	2.32 ± 0.21 (24)	10.3 ± 1.16 (27)	30.9 ± 1.59 (21)	10.4 ± 1.41 (13)	14.4 ± 1.39 (16)	[e]25.7 ± 1.9 (19)
Distal convoluted tubule	2.67 ± 0.20 (41)	1.10 ± 0.34 (3)	0.85 ± 0.27 (17)	7.43 ± 1.60 (9)	1.46 ± 0.30 (9)	10.3 ± 1.44 (12)	[f]5.21 ± 0.47 (33)
Medullary ray	—	—	0.66 ± 0.13 (21)	39.7 ± 2.88 (16)	—	—	—
High	2.55 ± 0.15 (4)	4.88 ± 0.43 (27)	—	—	3.85 ± 0.81 (3)	—	—
Low	3.08 ± 0.53 (5)	1.44 ± 0.39 (11)	—	—	12.5 ± 1.68 (10)	—	—
Medullary tubule Outer medullary zone	0.81 ± 0.13 (11)	0.36 ± 0.13 (16)	—	50.1 ± 4.50 (9)	[c]13.8 ± 1.32 (9)	6.4 ± 1.53 (10)	—
Inner medullary zone	1.27 ± 0.11 (8)	0.25 ± 0.11 (6)	—	[a]3.65 ± 0.68 (10) [b]3.20 ± 0.25 (10)	[d]1.40 ± 0.40 (12) 0.10 ± 0.037 (9)	8.7 ± 1.73 (10)	—
Papilla							
Base	1.15 ± 0.48 (6)	0.72 ± 0.27 (5)	0.63 ± 0.27 (3)	3.50 ± 0.27 (9)	0.11 ± 0.038 (10)	—	—
Apex	1.61 ± 0.49 (9)	0.27 ± 0.078 (11)	0.38 ± 0.11 (6)	3.88 ± 0.40 (10)	0.15 ± 0.027 (5)	—	—
Arteriole	1.12 ± 0.19 (25)	0.51 ± 0.097 (19)	0.76 ± 0.20 (12) 0.29 ± 0.090 (4) (capsule)	15.3 ± 0.69 (13)	2.72 ± 0.85 (5)	3.40 ± 0.60 (16) 0.66 ± 0.14 (8) (yellow tubules)	4.09 ± 2.52 (4) 2.80 ± 0.17 (18) (Wolff canal)

a dark, loop of Henle
b light, collecting tubule
c light, descending loop of Henle and collecting tubule
d dark, ascending loop of Henle
e. pronephritic nephron
f. mesonephritic nephron

* expressed in moles of substrate split per kilogram (dry weight) of tissue per hour
† number of individuals examined
‡ mean ± standard error of mean
( ) number of specimens analyzed

TABLE IX. Alkaline Phosphatase and Lactic Dehydrogenase Activity in Three Serial Biopsies of Patient LaC. Ill with Systemic Lupus Erythematosus and Treated with Prednisone *

	Alkaline Phosphatase			Lactic Dehydrogenase		
	Biopsy I	Biopsy II	Biopsy III	Biopsy I	Biopsy II	Biopsy III
omerulus	0.54 ± 0.14 (7)†	0.33 ± 0.030 (4)	0.21 ± 0.078 (3)	119 ± 9.9 (8)	114 ± 22 (4)	29.3 ± 1.05 (7)
ximal convoluted tubule	2.28 ± 0.56 (14)	2.30 ± 0.31 (9)	1.69 ± 0.45 (11)	394 ± 24 (8)	300 ± 14 (7)	98.4‡ ± 3.21 (6)
roximal degenerated tubule				272 ± 38 (5)	271 ± 21 (6)	112.3¶ ± 13.9 (7)
Distal convoluted tubule	1.73 ± 0.25 (7)	1.20 ± 0.40 (4)	1.37 ± 0.53 (4)	334 ± 43 (8)	244 ± 18 (5)	—
Medullary ray	—	1.79 (1)	1.32 ± 0.42 (3)	—	289 ± 16.5 (5)	77.9 ± 4.07 (8)
Vessel	1.31 ± 0.26 (6)	—	0.60 (1)	180 ± 10.3 (5)	—	36.6 ± 2.79 (3)

* expressed in moles of substrate split per kilogram (dry weight) of tissue per hour
† mean ± standard error of mean
‡ dilated
¶ fibrotic
( ) number of specimens analyzed

TABLE X. Hydroxyproline Content of Human Glomeruli and Small Renal Vessels

	Hydroxy-proline Per Cent Dry Weight	Collagen* Per Cent Dry Weight
Normal human glomeruli		
Female, 26 years	1.20	8.9
Male, 65 years	1.16	8.6
Male, 25 years — dissection	1.19	8.8
Male, 25 years — homogenate screening	2.50	18.5
Diseased human glomeruli		
Fanconi syndrome (adult)	1.57	11.6
Nephrotic sclerosis	1.65	12.2
Diabetic nephropathy	2.34	17.3
Normal renal vessels		
Female, 26 years	3.46	25.6
Male, 65 years	4.19	31.0
Male, 25 years	4.99	36.9

* Collagen contents were calculated on the basis of 13.5 per cent hydroxyproline in collagen

fore, we determined collagen in glomeruli obtained from the same kidney by Krakower's method and by dissection from frozen-dried sections. The results were 18.5 per cent for glomeruli isolated with the wet technique and 8.8 per cent for those dissected from dry sections. This indicates a considerable amount of solubilization with the wet technique. Further experiments are being done to investigate the discrepancy between our own and Krakower's results

Conclusions

(1) Enzyme activities can be estimated quantitatively and reproducibly in the individual anatomic and functional units of the nephron from renal biopsies of man in health and disease.

(2) Advantages of the quantitative histochemical approach are

(*a*) The treatment of the tissue preserves the enzyme activity very well. Results for alkaline phosphatase may be 50 times as high as after fixation and paraffin embedding.

(*b*) The specimen is dry until incubation, precluding the possibility of diffusion artefacts.

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Vessel	1.31 ± 0.26 (6)	—	*0.60* (1)	*180 ± 10.3* (5)	—	36.6 ± 2.79 (3)

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CONCLUSIONS

(1) Enzyme activities can be estimated quantitatively and reproducibly in the individual anatomic and functional units of the nephron from renal biopsies of man in health and disease.

(2) Advantages of the quantitative histochemical approach are,

(*a*) The treatment of the tissue preserves the enzyme activity very well Results for alkaline phosphatase may be 50 times as high as after fixation and paraffin embedding.

(*b*) The specimen is dry until incubation, precluding the possibility of diffusion artefacts.

(c) Long-term storage of tissue is possible without loss of enzyme activity (up to 1 year at −35° C. *in vacuo*), allowing additional enzyme assays to be made later on the same specimen.

(d) Almost any enzyme can be assayed specifically and accurately.

(e) There is close approximation of optimal conditions during assay.

(f) A very wide range of activities can be assayed in one specimen. In the case of the developing hamster tooth a 660-fold range was determined in one specimen.

(3) There is no correlation in many instances between histologic damage to tubules and levels of enzyme activity in those tubules.

(4) The level of alkaline phosphatase activity in the proximal tubules of a patient with hypophosphatasia was about 4 per cent of that in healthy proximal tubules and was 8 per cent of that in a patient with renal glycosuria. As the patient with hypophosphatasia did not have glycosuria, this observation suggested that alkaline phosphatase is not a key enzyme for the process of tubular reabsorption of glucose.

(5) Significantly low levels of both alkaline phosphatase and lactic dehydrogenase were found in all parts of the nephron only in Fanconi syndrome. This suggests a nonspecific effect of Fanconi syndrome in lowering enzyme activity in the nephron.

Summary

It has been shown that accurate quantitative enzyme analyses can be made on small fragments of renal tissue containing from 10 to 200 cells from identified structures (e g. proximal tubules).

The manipulation and handling of tissue preparatory to enzyme assay by different recognized techniques was investigated It was found that the results vary considerably from technique to technique. The highest levels were found with frozen-dried sections as used in our laboratory.

Data on the enzyme activities of the various portions of the nephron have been obtained for a wide variety of laboratory animals, including monkey, dog, rat, rabbit, frog, and toadfish, as well as man.

In potassium-depleted rats abnormalities were found in lactic dehydrogenase activity, and in the morphology of the collecting tubules studied by light and electron microscopy, which strongly suggest that the primary locus of the lesion involves the tubular cells in the papillary area

In disease states in man, abnormalities of enzyme activity were found in cases of the following diseases studied: systemic lupus erythematosus, rheumatoid arthritis, Fanconi syndrome, hypophosphatasia, renal glycosuria, and cystinosis Enzyme abnormalities were found in various units of the nephron which appeared histologically normal. Studies in man

augmented by studies on phlorizined dogs indicate that alkaline phosphatase and lactic dehydrogenase are not key enzymes in tubular absorption of glucose. Generalized depression of enzyme activity in adult Fanconi syndrome is considered to be secondary. The collagen content of the human glomerular tuft in health and disease was determined.

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A General Survey of Localization of Function Within the Nephron

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Studies in recent years have added considerably to our knowledge of the distribution of function in the mammalian nephron. In part these newer studies have confirmed earlier suppositions based upon analogy with lower forms or upon inferences not previously open to more direct confirmation. In part they have turned up, in certain segments, activities which these segments had not previously been suspected of possessing

In reviewing the recent growth and present status in this field it is pertinent to consider the methods which have been applied to the problems and their potentialities and limitations. Certainly the most powerful procedure for studying the localization of function is the micropuncture technique originally introduced b. Richards and his associates[14] The original extensive studies of functi in the amphibian still form the framework upon which most current ...els of the nephron are built. Fortunately, except for those proce... ssociated with the formation of hypertonic urine—a capacity n... esent in the amphibian kidney—the distribution of function in the ... al has shown few departures from that in the amphibian Few ol... extrapolations from the amphibian have, therefore, gone far fron ...nk The studies of Walker, Bott, Oliver, and MacDowell[37] exten... micropuncture technique to the mammal in what must be consi... preliminary way. Much of the recent progress has been due to ...nsion of these studies by Wirz[32] and Gottschalk,[8] particularly w... d to the distribution of the reabsorption of water and the bul... e Closely related to this method is the technique of Ullrich and h... ites[5] involving retrograde catheterization of the collecting ducts ... uster and collection of fluid from several levels of the collecting ... Application of this procedure has revealed previously unsuspected ... ties in the collecting ducts

The tremendous value of th... ethods for supplying definite information on localization is obvious Also obvious is the greatest disadvantage—the demanding nature of the technique We should also consider, however, the other limitations First, there is the fact that the

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II

*Morphologic Expression of Functional Changes in the Nephron**

E M. DARMADY, M A., M D, F R C P, and FAY STRANACK, B Sc.
(Portsmouth, England)

During the past decade physiologists have been active in determining the functions of the various segments of the nephron It is therefore important for the morphologist (whether he be anatomist or pathologist) to test the validity of these theories Unfortunately, histologic preparations do not allow a study to be made of the nephron in continuity, and for these reasons we have to supplement our studies by two techniques. microdissection,[8] and autoradiography of the isolated nephron [9] From microdissections we can make a precise examination of the nephron, and can determine the changes in the arrangement of the epithelium, which are well defined From this it is reasonable to suppose that such a change is likely to be correlated with a change in function [14] Furthermore, any disease which affects a single component will result in abnormal renal function [18] Autoradiography of the isolated nephron makes it possible to determine the location of various labeled compounds in the kidney

For example, when we examine the nephron we see that the proximal convoluted tubule surrounds its own glomerulus, and that it is divided into two portions, the convoluted portion and the lower end which is almost straight. By microdissection we can see the precise pattern of the epithelium. As far as the function of the proximal convoluted tubule is concerned, it is usual to accept that some of the functions of the proximal convoluted tubule in mammals and man are the reabsorption of glucose, the reabsorption and excretion of sodium, chloride, amino acids, phosphate, the production and excretion of bicarbonate, and the excretion of potassium Of these, only that of glucose reabsorption can be fully substantiated on morphologic grounds.[15] More recently Oliver [15] has been able to correlate the glucose Tm in man with the glomerular tubular volume,[15] and this has been confirmed by Bradley and Oliver [3] in dogs, thus exactly correlating function with morphology How this transfer is obtained

* This work is supported partly by the Medical Research Council of Great Britain, and partly by a grant (HTS 5323) from the National Heart Institute, United States Public Health Service.

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12

Observations on the Intrarenal Pressures

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Since a comprehensive survey of the physiologic and possible pathologic significance of the hydrostatic pressures in the various structures of the kidney is obviously impossible in the short time available, I will limit my remarks this afternoon to a few selected topics. My remarks must also be highly selective, for our knowledge of many of these parameters is quite fragmentary.

Take for example the glomerular capillary pressure. Largely as a result of the work in the laboratory of Dr A. N. Richards over a period of many years, it is generally believed that the initial step in urine formation in glomerular kidneys is the physical process of ultrafiltration at the glomerulus. The glomerular filtration rate thus must depend on the effective filtration pressure, which is the glomerular capillary pressure minus the sum of the pressure in Bowman's capsule and the colloid osmotic pressure, as well as the permeability of the glomerular membranes. The glomerular capillary pressure has been more or less directly determined in the frog[8] and Necturus,[14] but it is highly hazardous to transfer these findings directly to the mammalian kidney. This is obvious when one stops to consider differences in the kidneys of the Amphibia and Mammalia in respect to their vascular system, tubular morphology, etc. There are other very indirect estimates of the glomerular capillary pressure in the mammal,[4, 6, 15] but one must conclude that its magnitude and possible variations remain to be established.

A great deal more is known about the pressures in the proximal and distal tubules and peritubular capillaries of the rat kidney, however, since it has proved possible by micropuncture techniques to measure these pressures directly.[8, 7, 18] I should like to describe some of these relations in detail as they may offer some slight insight into the function of the kidney in disease as well as health.

In the anesthetized rat or other small mammal, one can visualize microscopically the convolutions on the surface of the kidney and, with suitable micromanipulative technique, puncture individual tubules. Following micropuncture, the mean intraluminal pressure can be accurately determined by measuring that applied pressure at which dye does not leave

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13

Pyelovenous Communications. A Functional Study

R. DOMINGUEZ, M.D., and R. B. ADAMS, B.S., M.S.
(Cleveland, Ohio)

A detailed study of the excretion of radioactive Diodrast was carried out by the following technique. An arteriovenous shunt was prepared on the femoral vessels of the dog and the blood, after heparin injection, was made to circulate in several turns of teflon tubing around a scintillation crystal. The gamma rays detected were counted by a scaler and the rate was computed in a counting rate meter. This rate was recorded continuously and automatically from the beginning of the injection until the end of the experiment. The mean arterial blood pressure in the other femoral artery and the mean shunt pressure on the return arm of the shunt were both measured with mercury manometers. The ureters were catheterized, the left as close to the pelvis as possible, and the catheters were fixed by silk ties (Figure 1).

For a study of Diodrast "extraction" during obstruction, the radioactive Diodrast was given by continuous intravenous injection and the left kidney was obstructed by the continuous intrapelvic injection of saline, both injections at a constant rate.[1]

For the direct observation of pyelovenous communications, the left kidney was obstructed by the continuous injection of radioactive Diodrast at a constant rate. In either case, the pressure developed in the pelvis by the injection of fluid was measured and recorded. The height of the reservoir was adjusted to give the required flow at the required position and at zero applied pressure. The reservoir was adjusted to maintain the hydrostatic head at the same height.[1] The right kidney was used as control. The mean rate of intrapelvic infusion was 0.077 ml. per minute (range 0.053 to 0.091). The urine flow from the control right kidney was on an average 0.160 ml. per minute (range 0.056 to 0.496).

In this paper we wish to present the observations made during obstruction, at decompression and during a 2-hour to 3-hour period after decompression.

The experiments with intrapelvic injection of radioactive Diodrast

and prognosis of a renal lesion. The other sort of correlation has to do with an examination of renal function, either normal or disturbed, as it may be explained on a structural basis.

The solution of these two very different correlative procedures requires, I believe, quite different techniques. For the first, that is, the diagnosis and prognosis of a renal lesion, the structural data are best obtained by the procedure of the histologic section, using material obtained either by post mortem or better by renal biopsy. For the second, where continuity of structure is essential for a comprehension of what is occurring functionally as the plasma fluid passes down the tubule to become urine, the structural data can only be obtained by microdissection of the nephrons that in their aggregate constitute the kidney

This dual nature of the technical problem is also observed when one considers the techniques of obtaining functional data for the correlative process. On the one hand, micropuncture gives exact and objective information concerning changes in the tubule fluid, and with the help of microdissection these changes can be accurately localized On the other hand, a variety of classical physiological techniques answer other sorts of renal questions.

We have heard reports today of important new contributions by all these methods of attack, Dr. Darmady's and Miss Stranack's demonstrations of localization in dissected nephrons of various substances by autoradiography and Dr Gottschalk's measurements of intraluminal pressure are examples of what may be called the direct approach to the examination of renal activity. Dr. Novikoff has shown the remarkable extension in our knowledge that has come from the application of histochemical procedures and electron microscopy, and in particular his combination of the two, to the examination of the histologic section Dr. Dominguez' and Dr. Adams' ingenious demonstration by radioactive tracers of the mechanisms of pyelovenous return represents the approach by elaborated classical physiologic methods

It would seem, therefore, that an aging renal morphologist might relax in his latter days in the beneficent climate of a world so ordered that every man knows what his job is and how he is to do it; where the laborers in the field are many and, even more important, the tools are at hand for garnering the harvest.

But for other than policemen an unclouded happiness is rarely the human lot, and as the morphologist looks about him he sees, or perhaps imagines, in the wealth and elaboration of both structural and functional investigation that today threatens to overwhelm us, dangers of a possible reaction similar to that of the earlier period of his unhappy youth when morphology was an unnecessary if not a suspect commodity. For as he is reading with great satisfaction Section I of Dr. Homer Smith's

"Principia" which has as its title *Anatomy – The Nephron*, he suddenly, on page 10, is shocked to find that those structural characteristics of the nephron which have so long been his professional delight abruptly suffer an alteration even more horrid than a sea change, nothing of their exquisite configurations but doth fade and there remains only something which, if not rich, is at least indeed strange, the bare bones of a "proximal" and "distal" segment or "tubule."

Now the reason for this simplification, which results from shutting one's eyes to obvious structural fact, is, we conclude, that the physiologist feels that he can make no finer distinctions. But rest assured a first-year student of *microscopic anatomy can do better, and to beg the entire* question of a forthright and meaningful correlation between the structural and functional characteristics of the nephron seems, at least to a morphologist, not only unnecessary but, in the pejorative sense, otiose.

And there is a further unfortunate aspect of the New Terminology, it has spread to those pathologic anatomists who limit their examinations to the use of histologic sections and who are, quite understandably at times, not certain of what they are looking at in a kidney whose normal and therefore identifiable anatomic characteristics have been transformed. And so by the operation of a sort of Gresham's Law where a worthless medium of exchange, in this case intellectual, rapidly replaces the more valid, the new terminology has caught on and it has now become impossible in reading or listening to an exposition of a problem involving the correlation of structure and function to know what is being meant as the old and still meaningful terms *proximal* or *distal convolution* alternate with the indeterminate new terms "proximal" and "distal tubule." I could cite from the literature occasions where third parties have had to disentangle the disputants at international conferences, who were involved in what can only be literally described as the new "double-talk," and I have friends so conditioned by the authoritative weight of the new terminology that even when they *have* punctured the *distal convolution* they hesitate to say more than that the sample comes from the "distal tubule."

But perhaps I am overstating the case, the term "distal tubule" does have certain advantages when one feels he must talk about something of which he is uncertain. In the current spate of studies by the urologists' elaborated two-glass test, what comes out first may perhaps more prudently be described as a sample from the "distal tubule." But where does the "distal *tubule" begin or end? Midway up the collecting ducts* at the junction of the inner and outer zone (for the two segments have quite a different structure), at the origin of the collecting tubule in the periphery of the cortex, does it include the distal convolution, one or both of the thin and thick limbs of Henle's loop or perhaps even the

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Significance of Chronic Pyelonephritis

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Pyelonephritis may be defined as an infectious disease of the kidney characterized by a primary inflammatory reaction of pelvis and parenchymal interstices to invading organisms with secondary effect on the tubular, glomerular, and vascular apparatus.

This concept permits a separation of pyelonephritis from other forms of interstitial nephritis which are pathogenetically different but which may histologically simulate pyelonephritis.

To assess the significance of chronic pyelonephritis, particularly the frequency with which it occurs and how often it results in uremia or possibly in hypertension, we must first clearly define the criteria used to establish a diagnosis. It would seem that by definition bacteriologic findings are of foremost importance but in reality we are not sure as yet of the meaning of true bacteriuria in its relationship to chronic pyelonephritis, and our experience with cultures of renal biopsy is still rather limited. Moreover, late stages are notorious for the frequent absence of a history of acute pyelonephritis.

We shall, then, examine the criteria for diagnosis based on structural changes in the kidney. The classical features established by the German authors[16] and by Weiss and Parker[17] in this country are generally accepted as reliable. As we look closer, however, we will discover many minor contradictions and some major discrepancies. I shall only mention a few of them. Vascular changes in chronic pyelonephritis are considered minimal and irrelevant by the German authors,[13, 16] whereas most of the American investigators stress their frequency and importance. The Germans describe a barrier zone as characteristic for chronic pyelonephritis, the Americans make no mention of it. Thyroid-like areas are described by all; some authors hold these to be pathognomonic, others do not. In the face of these uncertainties we must realize that what we know about the frequency and significance of chronic pyelonephritis is essentially based on autopsy findings irrespective of the availability of bacteriologic or clinical information. In fact, our confidence in pure morphology is so great that the existence of a noninfectious chronic pyelonephritis has been proposed,[13] and the assumption has been made that the inflammation

This incidence of pyelonephritis in our material is completely accidental since, during the period in which the material was collected, our interest in pyelonephritis has been rather variable. It is only for the last year that cases of pyelonephritis have been selectively chosen for biopsy.

The number of cases selected for study had to be further reduced because in some cases we did not find it possible to differentiate between the changes caused by pyelonephritis and changes caused by other coexisting disease. Thus 59 cases of acute renal failure were excluded. Similarly a number of cases of evident and severe pyelonephritis due to obstruction were excluded.

The reduced material contains 46 biopsies where the diagnosis of pyelonephritis was based on a variable combination of histologic and, in a few cases, clinical criteria

Before proceeding further I should like to present the clinical and histologic criteria with which we have worked. They probably do not differ much from those which are generally accepted in the United States

CLINICAL CRITERIA OF CHRONIC PYELONEPHRITIS

Acute pyelonephritis in history
Cystitis
Costovertebral angle pain and/or tenderness
Fever
Pyuria
Bacteriuria
Kidney function reduced
Positive x-ray

HISTOLOGIC CRITERIA OF PYELONEPHRITIS

Cell casts
Interstitial infiltration
Invasive glomerulitis
Periglomerular fibrosis
Tubular atrophy
Peritubular fibrosis
Dilated tubules with acellular casts
("thyroid-like")

The 46 cases were divided into three groups according to their kidney function expressed as endogenous creatinine clearance (Table I). Most of the patients were in the groups with low or very low kidney function. Four of 6 patients with normal kidney function had a history of acute

TABLE I DIVISION OF 46 PATIENTS WITH PYELONEPHRITIS BY GROUPS ACCORDING TO KIDNEY FUNCTION

24 hrs Endogenous Creatinine Clearance	Number of Patients	Acute Pyelonephritis in History	History of Excessive Phenacetin Consumption		
			+	0	?
>70 ml/min	6	4	4	1	1
20–70 ml/min	16	6	4	2	10
<20 ml/min	24	4	14	9	1

attacks of pyelonephritis, while only 4 out of 24 patients in the group with severely damaged kidney function had such a history. The 16 patients in the middle group occupied an intermediate position This is a peculiar and noteworthy but well-known observation. No significant histologic differences were observed between cases with and without a history of previous attacks of acute pyelonephritis

Another striking but not unknown observation is that approximately 50 per cent of the patients had for many years an excessively high intake of drugs containing phenacetin In the earliest biopsies no information on the phenacetin consumption was available, and in many newer records the statements were not expressed precisely enough to make an evaluation possible When asked why they had taken phenacetin, most of the patients declared that they took it because they had suffered from headache or, in fewer cases, backache We were not able to find any clinical or histologic changes which separated the patients who consumed phenacetin from those who did not.

Figures 1 to 10 show microphotographs of typical lesions found in renal biopsies from patients with pyelonephritis They illustrate the classical histologic changes listed above (See pages 235–239)

We have tried to determine to what extent there was a correlation between the biopsy findings, the clinical diagnosis, and kidney function.

The evaluation of our material is made difficult by the aforementioned fact that the clinical criteria are vague and ambiguous and the diagnosis has to be established on varying combinations of these criteria The histologic criteria are not much better, and probably neither interstitial infiltration nor periglomerular fibrosis is completely specific for pyelonephritis

The only way we found it possible to approach the problem was by reducing the number of patients still further If we omit all cases where any room for doubt can be found with regard to diagnosis and consider only biopsies from patients in whom the clinical diagnosis has been established on the most certain available criteria, it is possible to form an

tions, as in Table II, that the tissue removed by renal biopsy in pyelonephritis is to a large extent representative. This statement corresponds to the practical experience that the foci of cellular infiltrate or tubular atrophy in biopsies from cases of pyelonephritis generally are of such small dimensions that several such foci are found in one biopsy.

Therapeutic Value of Kidney Biopsy in Pyelonephritis

Culture from a renal biopsy may be a more accurate method than culture of urine in determining the species of bacteria responsible for infection within the kidney. This should increase the chance of getting positive therapeutic results after susceptibility tests.

So far we have only worked with a relatively primitive technique because we do not grind up a biopsy specimen and use it for culture studies. The proper technique is obviously to take two biopsies or cut one into two pieces under aseptic precautions. Our technique was simply to make cultures from the renal blood on the inside of the syringe which we use when securing the biopsy. The urine cultures were made by the traditional qualitative method. This gives no or, at best, only a very rough impression of the quantitative aspect and probably should be supplemented by bacterial counts as described by Kass.[7] In Table III the preliminary

TABLE III Results of Qualitative Cultures of Renal Biopsy Material Correlated with Urine Cultures *

	Renal Biopsy Culture	Urine Culture	Number with Biopsy and Urine Culture Agreement†
Positive	9	14	6
Negative	13	8	7

* From 22 patients with chronic pyelonephritis
† Same organism or no growth

bacteriologic results are presented. In 9 cases, or a little less than half, a positive culture from tissue was obtained. Only 1 of these cases had sterile urine, and in only 3 cases was more than one bacterial species found. In 13 cases agreement was found between the culture of tissue and urine, that is, either the same organism or no growth. It seems to us that even if our technique is not ideal, it is still of some value and we plan to continue to use it.

In still another aspect the biopsy has been of value in the clinical treatment of the patient.

Sometimes cases of chronic renal disease are sent to our acute renal failure ward because they are mistaken for acute renal failure, or because it is questionable whether the patient is in terminal renal failure or only in an acute aggravation with the possibility of regaining some degree of kidney function. In this situation a kidney biopsy may be of great value. We do not believe it reasonable or humane to dialyze patients who have no anatomic possibility of recovering. If the biopsy reveals chronic pyelonephritis in which some of the nephrons seem to have escaped destruction, we are generally more inclined toward active therapy. If the biopsy shows a nearly complete glomerular destruction, as in severe glomerular nephritis, we feel less inclined to employ hemodialysis therapy. Owing to the risk of heparinizing a patient immediately following a renal biopsy, we often feel it necessary to give the patient the benefit of the doubt and to do an initial dialysis. The result of the biopsy performed the day after the dialysis will then be decisive for the therapy chosen. A great amount of experience will be needed before such a decision can be made with sufficient certainty, but we believe it necessary and are attempting to collect observations which in future years can be used to this end. Table IV shows the degree of recovery of renal function which took place and the survival observed in our dialyzed cases.

TABLE IV KIDNEY BIOPSY AND HEMODIALYSIS IN 10 CASES OF PYELONEPHRITIS *

Biopsy No	Creatinine Clearance at Day of Biopsy	Maximum Creatinine Clearance	Minimal Survival Period
511	2 4 ml /min	7–8 ml / min	8 months
562	0 3	0 3	17 days
274	0 5	25–30	29 months
508	0	0	2 days
356	0 1	47	6 months
555	0 5	3 8	4 months
538	0 1	6 0	4½ months
453	0 9	2 0	1 month
336	0 1	41 0	2 months
358	0 7	53 5	6½ months

* Minimal and maximal kidney function observed, and minimal observed survival period from time of dialysis

Even in some undialyzed cases of chronic pyelonephritis in patients who were admitted *in a state which turned out to be acute aggravation*, the biopsy was at least of prognostic value. The biopsies showed that several glomeruli had escaped destruction. In correspondence to this the creatinine clearance gradually improved during a month or two. A biopsy from such a case is shown in Figure 10.

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bacteriologic results are presented. In 9 cases, or a little less than half, a positive culture from tissue was obtained Only 1 of these cases had sterile urine, and in only 3 cases was more than one bacterial species found In 13 cases agreement was found between the culture of tissue and urine, that is, either the same organism or no growth. It seems to us that even if our technique is not ideal, it is still of some value and we plan to continue to use it.

In still another aspect the biopsy has been of value in the clinical *treatment of the patient.*

Sometimes cases of chronic renal disease are sent to our acute renal failure ward because they are mistaken for acute renal failure, or because it is questionable whether the patient is in terminal renal failure or only in an acute aggravation with the possibility of regaining some degree of kidney function. In this situation a kidney biopsy may be of great value. We do not believe it reasonable or humane to dialyze patients who have no anatomic possibility of recovering. If the biopsy reveals chronic pyelonephritis in which some of the nephrons seem to have escaped destruction, we are generally more inclined toward active therapy. If the biopsy shows a nearly complete glomerular destruction, as in severe glomerular nephritis, we feel less inclined to employ hemodialysis therapy. Owing to the risk of heparinizing a patient immediately following a renal biopsy, we often feel it necessary to give the patient the benefit of the doubt and to do an initial dialysis. The result of the biopsy performed the day after the dialysis will then be decisive for the therapy chosen. A great amount of experience will be needed before such a decision can be made with sufficient certainty, but we believe it necessary and are attempting to collect observations which in future years can be used to this end. Table IV shows the degree of recovery of renal function which took place and the survival observed in our dialyzed cases.

TABLE IV. Kidney Biopsy and Hemodialysis in 10 Cases of Pyelonephritis *

Biopsy No.	Creatinine Clearance at Day of Biopsy	Maximum Creatinine Clearance	Minimal Survival Period
511	2.4 ml/min	7–8 ml/min	8 months
562	0.3	0.3	17 days
274	0.5	25–30	29 months
508	0	0	2 days
356	0.1	47	6 months
555	0.5	3.8	4 months
538	0.1	6.0	4½ months
453	0.9	2.0	1 month
336	0.1	41.0	2 months
358	0.7	53.5	6½ months

* Minimal and maximal kidney function observed, and minimal observed survival period from time of dialysis.

Even in some undialyzed cases of chronic pyelonephritis in patients who were admitted in a state which turned out to be acute aggravation, the biopsy was at least of prognostic value. The biopsies showed that several glomeruli had escaped destruction. In correspondence to this the creatinine clearance gradually improved during a month or two. A biopsy from such a case is shown in Figure 10.

tions, as in Table II, that the tissue removed by renal biopsy in pyelonephritis is to a large extent representative. This statement corresponds to the practical experience that the foci of cellular infiltrate or tubular atrophy in biopsies from cases of pyelonephritis generally are of such small dimensions that several such foci are found in one biopsy.

Therapeutic Value of Kidney Biopsy in Pyelonephritis

Culture from a renal biopsy may be a more accurate method than culture of urine in determining the species of bacteria responsible for infection within the kidney. This should increase the chance of getting positive therapeutic results after susceptibility tests.

So far we have only worked with a relatively primitive technique because we do not grind up a biopsy specimen and use it for culture studies. The proper technique is obviously to take two biopsies or cut one into two pieces under aseptic precautions. Our technique was simply to make cultures from the renal blood on the inside of the syringe which we use when securing the biopsy. The urine cultures were made by the traditional qualitative method. This gives no or, at best, only a very rough impression of the quantitative aspect and probably should be supplemented by bacterial counts as described by Kass.[7] In Table III the preliminary

TABLE III. Results of Qualitative Cultures of Renal Biopsy Material Correlated with Urine Cultures *

	Renal Biopsy Culture	Urine Culture	Number with Biopsy and Urine Culture Agreement†
Positive	9	14	6
Negative	13	8	7

* From 22 patients with chronic pyelonephritis
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bacteriologic results are presented. In 9 cases, or a little less than half, a positive culture from tissue was obtained. Only 1 of these cases had sterile urine, and in only 3 cases was more than one bacterial species found. In 13 cases agreement was found between the culture of tissue and urine, that is, either the same organism or no growth. It seems to us that even if our technique is not ideal, it is still of some value and we plan to continue to use it.

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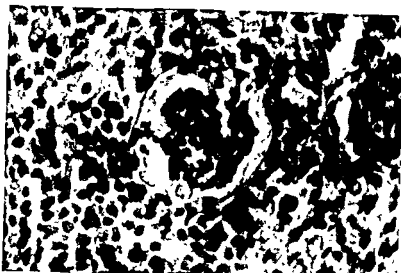


Figure 7. Biopsy 584. 57-year-old female. Chronic alcoholism and CVA-pain Cystitis symptoms? At admission anuria and uremia Kidney function zero [illegible]
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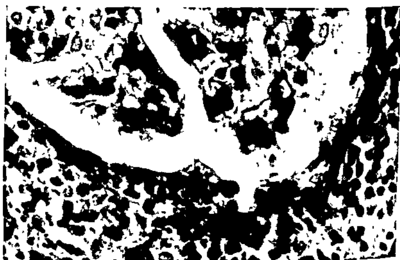


Figure 8 Biopsy 584 Same as Figure 7. Invasive glomerulitis Part of Bowman's capsules with defect in basal membrane and polymorphonuclear leukocytes in pericapsular lymph spaces Leukocytes are found on epithelial side of basal membrane, and early epithelial reaction is seen. PAS & hematoxylin stain X250



FIGURE 9 Biopsy 549 53-year-old female No history available when admitted in coma with uremia and hypertension (205/120 mm Hg) Creatinine clearance 5 ml/min No phenacetin consumption Gradual but incomplete recovery to creatinine clearances around 30 ml min in 6 weeks Biopsy shows severe chronic pyelonephritis Invasive glomerulitis in glomerulus (center) surrounded by cell infiltrate PAS & hematoxylin stain ×60

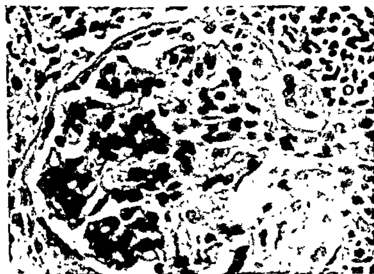


FIGURE 10. Biopsy 549 Detail of Figure 9 Localized epithelial reactions in Bowman's capsule ("crescent") Adhesion between glomerular tuft and Bowman's capsule PAS & hematoxylin stain ×95

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Suspected and Unsuspected Pyelonephritis in Renal Biopsies

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(Chicago, Illinois)

As a pathologist, my experience in renal diseases in general and of pyelonephritis in particular has developed first from the study of autopsy material and of surgically removed kidneys, and secondly, during the last seven years, from the study of percutaneous renal biopsies The value of percutaneous biopsies in the study of renal diseases is now well established. The major disadvantage of this procedure, of course, is the limited amount of tissue available In the histologic evaluation of renal biopsies, it is imperative therefore that a most careful analysis of the various changes be carried out and that every single abnormal feature be utilized in an attempt to reach a diagnosis or at least to provide the clinician with useful information The limitations and difficulties inherent in the interpretation of renal biopsy specimens are particularly great in pyelonephritis since none of the changes occurring in this disease can be considered characteristic when considered individually Only a combination of these changes is diagnostic, especially in the absence of lesions due to other renal diseases In addition, the focal or patchy character of pyelonephritis and, in most instances, the frequent absence of renal medulla make the definitive diagnosis particularly difficult except for the more severe and diffuse forms of this disease.

In acute pyelonephritis, no diagnostic problems usually arise The presence of neutrophils in the interstitial tissue and within the tubules in the absence of significant glomerular abnormalities is considered diagnostic. In some cases, neutrophils accumulate around Bowman's capsule and eventually breach it, invading the glomerular space Histologically, acute exudative glomerulonephritis may simulate this picture (Figure 1) and a careful study of the glomeruli is always necessary In this respect, the possibility of acute bacterial glomerulonephritis should also be mentioned. In this period in which hypersensitivity is brought forth to explain the pathogenesis of every inflammatory disease of the glomeruli, the view that bacteria can act directly on the glomerular tufts is not likely to meet with much approval. However, bacterial glomerulitic lesions do

occur in severe bacteremias and, in a transient form, may actually be part of the early phases of acute hematogenous pyelonephritis. Differences in bacterial species, routes of infection and local factors are, of course, important to explain the predominant interstitial localization of micro-



FIGURE 1 Acute exudative and necrotizing glomerulonephritis simulating acute pyelonephritis Note the numerous polymorphonuclear leukocytes within and around the tubules In other areas (not shown), necrotizing glomerular lesions were seen Autopsy specimen H & E × 120

organisms. Among the local factors, mention should be made of the differences in pressure and velocity of the blood and in capillary wall structure

In chronic pyelonephritis, histologic diagnosis is considerably more difficult and often uncertain. When interstitial fibrosis with round cell infiltrates, Bowman's capsular fibrosis, atrophy and dilatation of tubules with colloid casts are all present in the sections, a reasonably certain diagnosis can be entertained (Figure 2). However, not all these features are always present or necessarily contained within the limited amount of tissue available (Figure 3). Indeed, Kipnis and co-workers[6] reported they had found essentially normal renal tissue in 4 of 13 biopsy specimens from patients in whom clinical and laboratory findings suggested the presence of pyelonephritis. In a larger series of similar cases, Jackson *et al.*[4] found normal renal tissue in 25 per cent of the biopsies. The diagnostic importance of the vascular changes in pyelonephritis, in my opinion, is not too great. It is true, as indicated by several observers,[1, 9]

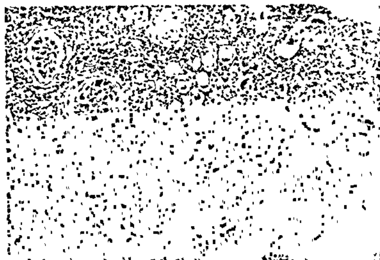


FIGURE 2 Chronic pyelonephritis Note all the classic features of this disease interstitial fibrosis with round cell infiltrates, Bowman's capsular fibrosis, atrophic tubules with colloid casts (thyroid-like appearance) and sclerosis of small arteries The glomerular changes are secondary and nonspecific Biopsy H & E ×110

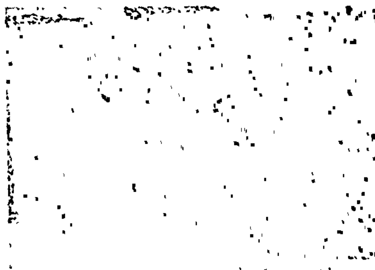


FIGURE 3 Chronic pyelonephritis in a [illegible]-year-old white man with malignant hypertension of 1-year duration There is a moderate interstitial fibrosis and inflammation The profound tubular atrophy is more consistent with pyelonephritis than with malignant hypertension The tortuous and fibrotic interlobular arteries exhibit features of both diseases Biopsy H & E ×120.

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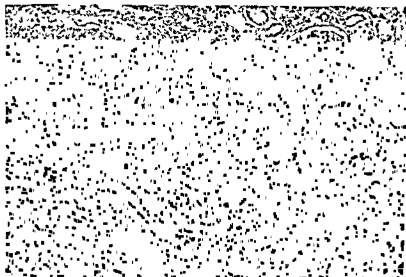


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F[illegible] pyelonephritis Note all the classic features of this disease in[illegible] fibrosis with round cell infiltrates, Bowman's capsular fibrosis, atrophic tubules with colloid casts (thyroid-like appearance) and sclerosis of small arteries The glomerular changes are secondary and nonspecific Biopsy H & E ×120



Figure 3 Chronic pyelonephritis in a 37-year-old white man with malignant hypertension of 1-year duration There is a moderate interstitial fibrosis and inflammation The profound tubular atrophy is more consistent with pyelonephritis than with malignant hypertension The tortuous and fibrotic interlobular arteries exhibit features of both diseases Biopsy H & E ×120

that these changes in the pure form of this disease are different from those of arteriosclerosis. However, a considerable number of cases of chronic pyelonephritis are complicated by hypertension and, as a result, intimal lamination and elastic reduplication are superimposed on the intimal



FIGURE 4 Severe hypertension in a young woman. The histologic findings are consistent with chronic pyelonephritis Note the marked interstitial fibrosis and the numerous round cells. The small and tortuous interlobular artery exhibits intimal fibrosis Biopsy. H & E ×120

fibrosis and medial atrophy usually seen in the narrow tortuous vessels of chronic pyelonephritis (Figure 4). Actually, in many cases, it is extremely difficult if not impossible to say whether a cortical scar is due to healed pyelonephritis or to arteriosclerosis.

One problem that has been of considerable interest to us is the presence of pyelonephritis complicating other renal diseases. During the past three years, 1956–1958, 258 percutaneous renal biopsies were performed at the Research and Educational Hospitals by Dr R. M. Kark and his associates on patients in whom renal diseases other than pyelonephritis were suspected on clinical and laboratory grounds. Histologically, in 16 cases or a little more than 6 per cent, pyelonephritis was either definitely diagnosed or strongly suspected. In many of these cases, the diagnosis was later confirmed either by repeated urine cultures or by the beneficial results of antibiotic therapy. Seven of the 16 patients had only pyelonephritis and 9, or slightly more than 3 per cent, were found to have pyelonephritis superimposed on other renal diseases such as diabetic

nephropathy, arteriosclerosis, glomerulonephritis, and lupus nephritis. In the absence of obvious obstruction and lower urinary tract infection, these cases of pyelonephritis were considered to be of the hematogenous type. A 3 per cent incidence indicates the great value of renal biopsy in



FIGURE 5 Diabetic glomerulosclerosis with probable superimposed chronic pyelonephritis. Note the round cell infiltrate and the colloid casts. Biopsy H & E ×145

revealing "unsuspected" pyelonephritis, and also supports the view that previous renal disease predisposes to renal infection and the development of pyelonephritis.

In patients with diabetes mellitus, pyelonephritis has been reported in the past to be as much as four times more frequent than in nondiabetics[a] My experience and that of others[2, 3] point to a considerably lower incidence, at least in recent years and especially in biopsy material. This probably indicates the effectiveness of antibiotic treatment as well as the limitations of renal biopsies in the diagnosis of pyelonephritis. In one such case, the histologic changes were not entirely convincing, pyelonephritis was suspected but not definitely diagnosed (Figure 5). I should say here that, no matter how slim the histologic evidence of pyelonephritis, it is essential that the pathologist mention this possibility in his report. By doing so, additional laboratory studies can be undertaken and effective antibiotic therapy instituted to prove or disprove this diagnosis.



FIGURE 7 Interstitial fibrosis and tubular atrophy in a patient with chronic hypopotassemia and clinical pyelonephritis Biopsy. H & E ×110

pyelonephritic lesions but also because of the limited amount of tissue available. As long as the pathologist and the clinician are aware of these limitations, renal biopsies in association with careful clinical and laboratory studies can contribute greatly to the understanding of pyelonephritis per se as well as of the role that pyelonephritis plays in other renal diseases.

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The Evolution of the Experimentally Produced Pyelonephritic Lesion

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(London, England)

The growing realization that chronic pyelonephritis is an important cause of renal failure and high blood pressure makes it imperative that we fully understand the evolution of this condition. Apart from increasing our information on the nature of the disease, a detailed knowledge of the various pathological features is essential for accurate diagnosis, and the current interest in the percutaneous renal biopsy as a means of diagnosing renal disease emphasizes this fact. The frequent presence of severe arteri[illegible]
has recently [illegible]
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considered that in the stage of acute infection there was an acute arteritis which healed with the production of considerable intimal thickening. According to this concept the first change in the evolutionary cycle is an atrophy of proximal convoluted tubules without changes in the glomeruli, a picture identical with that seen in kidneys with severe obstruction to their blood supply. It is a picture seen with great consistency in kidneys removed at operation for the treatment of hypertension due to renal artery obstruction. The next stage is the appearance of chronic inflammatory cells and some degree of periglomerular fibrosis, and this shows transitions to the more familiar picture of chronic pyelonephritis in which hyalinized glomeruli are crowded together with great atrophy of tubules, and chronic inflammatory cells are present in the interstitium. The dilated tubules containing eosinophilic casts, the so-called thyroid-like areas, were considered to be a separate change, the direct result of acute inflammation. This thesis stands in contrast to the more conventional view that scarring is largely the end-result of the damage caused by the acute inflammatory process.

The prime object of this experiment was to investigate the role of vascular obstruction in the production of the atrophic kidney of pyelonephritis in rabbits. New Zealand white rabbits of approximately 2.5 Kg

were given an intravenous injection of *Bacterium coli* (100×10^6 organisms in a 4-hour shake culture per kilogram of body weight) shortly after complete occlusion of the left ureter. After 3 days the ureteral occlusion was relieved and continuity of the ureter was restored[1] At various time intervals of 12 hours to 9 months following the injection of organisms the animals were killed and the kidneys injected with a suspension of barium Radiographs of both kidneys were prepared after removal from the body, using fine-grain paper. After fixation, histologic preparations were made from both kidneys.

Examination of radiographs of the arterial tree of the normal kidney showed filling as far as interlobular arteries (Figure 1). Study of the infected kidneys showed that at no stage up to 9 months was there any obstruction of arteries down to arcuate size, and the only detectable abnormality was an increased tortuosity of the vessels as reduction of parenchyma occurred Interlobular arteries failed to show a clear pattern during the acute stage, but at 14 days and subsequently they were visible, differing from those in the control kidneys in that they were shorter because of cortical reduction and pursued more angulated and tortuous courses (Figure 2). At all stages good filling of arteries and arterioles was found histologically and there was no intimal thickening or other change in the vessel walls. In 2 out of 8 rabbits at 6 months or later a few scattered arteries showed a mild eccentric intimal thickening which was so trivial that there was no interference with the filling of the vascular tree beyond

Macroscopically, infected kidneys showed an increase in size, up to three times normal at 7 days, with numerous small cortical abscesses. Thereafter they decreased, so that by 2 months they were the same weight as the control and at 9 months were reduced to as little as one-third of the control. The contracted kidneys showed either a diffuse reduction in cortical width with smooth subcapsular surfaces or a patchy scarring with depressed smooth areas between apparently raised areas (Figure 3)

Microscopically it was possible to trace the sequence of events from the early acute stage through to the scarred end-picture In a communication as brief as this one only relevant points will be mentioned, a fuller account is given elsewhere[2] The earliest lesions were found at 12 and 24 hours and consisted of patchy foci of dilated capillaries stuffed with polymorphs and containing organisms, in all zones of the kidney, particularly the papilla At 24 hours collections of proximal convoluted tubules, in relation to the infected capillaries described, showed increased eosinophilia of their cytoplasm with early nuclear loss (Figure 4) This change is particularly significant as it represents a change which plays an important role in parenchymal loss. At 3 and 7 days acute

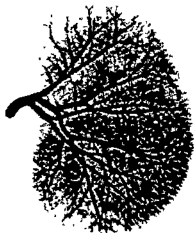


FIGURE 1 Radiograph of normal right kidney showing filling as far as interlobular arteries

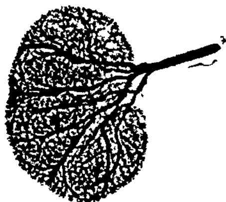


FIGURE 2 Radiograph of kidney shown in Figure 3 (5 months after infection) There is some tortuosity of larger arteries, and interlobular arteries are well filled although irregular in direction.



FIGURE 3. Kidney with irregular scarring 5 months after injection of organisms.

abscesses were present in all parts of the kidney, and in these there was complete disappearance of tubules, with some glomeruli showing acute inflammatory changes and others showing no change. In the intermediate and medullary zones small foci of acute inflammation in the interstitium showed ruptures into tubules, mainly collecting tubules, many of which were filled with polymorphs. In areas surrounding the cortical abscesses there was a diffuse infiltration with polymorphs and at 7 days an admixture of lymphocytes. Groups of proximal convoluted tubules showed necrotic changes such as were seen at an early stage in the 24-hour animals, while others were atrophic or had disappeared. Glomeruli in these areas were normal and there was edema of the interstitium. Organisms were easily demonstrable. Certain areas in the kidney were perfectly normal. At 14 days abscesses were smaller but the surrounding areas showed considerable loss of proximal convoluted tubules, with persistence of collecting tubules and other simple tubules lined by low cubical epithelium. Glomeruli, apart from being crowded together, were normal and the interstitium showed a diffuse infiltration with lymphocytes and smaller numbers of plasma cells. Many tubules still contained

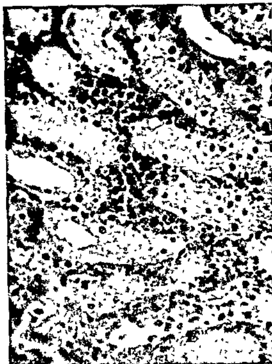


FIGURE 4 24-hour infection Intertubular capillaries are stuffed with polymorphs, and proximal tubules show swelling of cytoplasm and some nuclear loss H & E ×320

polymorphs but organisms were difficult to demonstrate At 21 and 28 days the few abscesses which remained were composed almost entirely of lymphocytes with some adjacent fat-filled macrophages In the surrounding areas tubular loss was if anything greater (Figure 5), and in addition there was hyalinization of occasional glomeruli Lymphocytes and plasma cells were still present in large numbers and a fine increase in collagen was apparent. In the intermediate zone, tubules still contained polymorphs but eosinophilic casts were now appearing At 2 months the lesions had progressed, so that increasing numbers of glomeruli in the areas around abscesses showed abnormalities in the form of either periglomerular fibrosis or hyalinization of the whole tuft, sometimes with a surrounding mantle of collagen. Interstitial lymphocytes and plasma cells were still plentiful and collagen formation was increased Very few tubules now contained polymorphs, and casts were plentiful. At 4 and 5 months glomerular

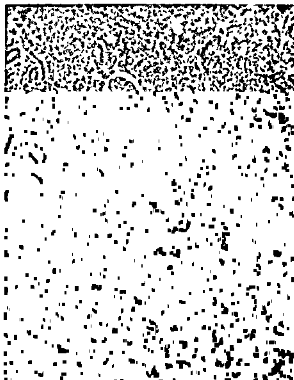


FIGURE 5 28-day infection There is intense loss of proximal convoluted tubules and persistence of collecting tubules with some other tubules lined by low cubical epithelium Glomeruli are little changed and there is some lymphocytic infiltration H & E ×140

hyalinization was conspicuous (Figure 6), inflammatory cells were decreased, and more tubules were dilated and contained eosinophilic casts Some kidneys showed very little glomerular hyalinization, and apparently normal glomeruli were found in company with great tubular loss (Figure 7) It was noticed that the greatest amount of glomerular hyalinization was present in association with the greatest interstitial cellular response. At subsequent times up to 9 months glomerular changes increased although some kidneys, while showing profound tubular loss, showed very little glomerular abnormality Interstitial fibrosis was now much greater, but inflammatory cells showed a progressive decrease, so that at 9 months they were sparse Eosinophilic casts in dilated tubules bearing a certain resemblance to thyroid tissue were increased. Certain areas of the parenchyma were unchanged at all times

It is apparent from what has been said that severely contracted kidneys may be produced in the absence of arterial changes of structural type

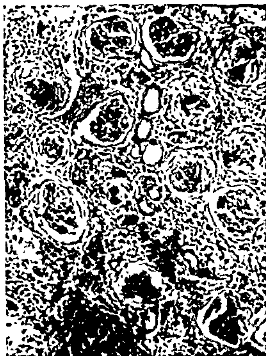


FIGURE 6 4-month infection Some glomeruli completely hyalinized and others showing early periglomerular fibrosis There is much tubular loss and some lymphocytic infiltration Arterioles can be seen well filled with barium H & E ×140

At no stage in the production of the scarred kidney was there appreciable arterial change. This accords well with the findings in a previous experiment[1] and with those of Mallory, Crane, and Edwards,[4] who, although finding occasional vascular changes, commented that vascular changes were not prominent. While injection of the kidneys by distending the arterial tree could conceivably smooth out minor degrees of intimal thickening, it is considered that intimal thickening sufficient to cause parenchymal loss would be readily detectable even in injected tissues Examination of human chronic pyelonephritic kidneys injected with barium at much greater pressures than those used in the present experiment shows very obvious intimal thickening Consideration of the histologic sequences suggests that the direct effect of the causative organism and the accompanying inflammatory process are responsible for the parenchymal loss The acute abscesses are quite clearly associated with destruction of



FIGURE 7. 5-month infection Considerable tubular loss in association with normal glomeruli Arteries and arterioles well filled with barium and showing no intimal thickening. Cellular reaction slight H & E ×100

parenchyma and, although they decrease in size with time, can still be recognized as severely fibrotic areas in which tubules are completely absent and in which there are severely scarred glomeruli. The zones of less intense inflammation which surround acute abscesses are those of greater interest and importance. In these, necrosis of proximal convoluted tubules is obvious at a very early stage and this is followed by a more gradual atrophy over a period of months It is considered that much of the tubular damage is the direct result of bacterial endotoxin, while the general inflammatory response is no doubt responsible for loss of tubules other than those showing actual necrosis. The way in which the tubular loss becomes permanent and progressive as the lesion ages can perhaps best be explained by Hinman's concept of renal counterbalance[8] On this basis it would be necessary to assume that the opposite noninfected kidney takes over most of the work of the infected kidney, whose function must be considerably deranged during the acute stage. Because of this trans-

fer of work from the sick to the healthy kidney there is no stimulus for the infected kidney to recover, so that tubular regeneration would not occur and tubules already partly damaged would undergo atrophy. Additional evidence against the likelihood of arterial damage being responsible for scarring is provided by the presence of considerable tubular loss long before intimal thickening could play any causative role.

In summary, it might be stated that scarring of experimental chronic pyelonephritis may be produced in the absence of organic arterial disease.

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18

*Hereditary Chronic Nephritis**

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In previous reports from this laboratory the clinical manifestations, genetics, and pathology of hereditary chronic nephritis in a large family were described.[1,2] Data from 217 members of the family, most of whom were examined during the course of the study, were available for analysis. Ten men had become deaf and died in uremia. In addition, 74 persons had abnormal findings in the urine. Of 86 audiograms done in patients other than the 10 who died, 21 revealed nerve deafness. Usually, but not always, the deafness occurred in patients who had evidence of renal disease. Most patients were asymptomatic early in life, but some had periodic episodes of fever and pyuria. The males usually developed chronic renal insufficiency and died before they reached 40 years of age. Two autopsies and five percutaneous needle biopsy specimens revealed variable degrees of renal inflammation and glomerular lesions. Lipid-laden foam cells were found in some of the sections. Small numbers of foam cells sometimes could be found in otherwise unremarkable biopsy specimens obtained from young patients with abnormal urinalyses. The pattern of inheritance was compatible with that of a partially sex-linked dominant trait.

Examples of hereditary renal disease have been reported sporadically for many years, and in a recent paper describing a new family, the old literature was reviewed thoroughly.[4] Throughout the years, different authors have described the disorder as interstitial nephritis, glomerulonephritis, pyelonephritis, and most recently[illegible] as a disorder with histologic features of all of these renal diseases. The purpose of the present paper is to compare the clinical, histologic, and genetic findings in several of the reported families from which adequate data are available[illegible] and to discuss some of the problems that have arisen in relation to this interesting disease.

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Clinical Features of the Disease

In the initial study of our group of patients, abnormal urinary sediments were found in 44 patients Of these, only 2 had symptomatic disease at that time, although some had had episodes of genitourinary tract infection previously. Both of the symptomatic patients were young adult males with advanced renal disease who subsequently died in uremia. In the next six years, several of the asymptomatic patients had episodes of pyuria, fever and bacteriuria, which responded to appropriate antibiotic or chemotherapy. In a later study of the same family, 49 of 168 persons examined had abnormal urinalyses. Except for a few patients who had intermittent costovertebral angle tenderness, nocturia, or dysuria, the majority were asymptomatic. In addition to the severely involved males, one woman, age 62, had died, presumably of the same disorder as that which affected other members of her family.

This general clinical pattern is reproduced, with minor differences, in all of the families selected for review. In a report published in 1927, further studies of a family previously known to have hereditary renal disease revealed that in 16 involved members, intermittent hematuria was the only symptom found, even though urinalyses were grossly abnormal.[7] Involved males, however, later developed chronic renal insufficiency and died in uremia, whereas females remained well. The association of nerve deafness with hereditary renal disease was noted for the first time.

More recently studies of two other groups of patients with hereditary renal disease yielded similar clinical findings.[9] Males became deaf and died with progressive renal insufficiency, while involved females were asymptomatic As in our group of patients, some of the females in the first of these two families also showed nerve deafness by audiometry The patients in the second family, moreover, had periodic dysuria, pyuria, fever, and other evidences of genitourinary tract infection

Two additional groups of patients are similar.[4, 12] In the former, two of the four subjects were recognized to have renal disease only in the late stages of its course, shortly before death. One male, except for deafness, was relatively well. In the latter family, intermittent hematuria was the usual early symptom, with deafness and progressive uremia only in the males.

Associated Clinical Findings

In two of the families under review, abnormalities of the eye have been noted in some of the involved patients.[4, 8] In one, anterior subcapsular cataracts were present in one of four patients.[4] In the other, unspecified

"eye disease" occurred.[8] In two other groups, not included for detailed discussion because of lack of adequate histologic or genetic findings, lenticular anomalies[10] or abnormalities of the integument or bone[4] also were noted. These findings have not been present in other families, and their significance is unknown.

Laboratory Findings

A high incidence of pyuria, with cylindruria and proteinuria being prominent but less common features, was found in our patients. Of special interest in relation to the other families reported is the observation that microscopic hematuria was almost as common a finding as pyuria. In one other group, pyuria was common.[8] The remainder of the families showed hematuria and proteinuria as the major urinary abnormalities.

Studies of blood urea, electrolytes, proteins and, in a few instances, lipids have been unrevealing. In two families in which the studies were performed, urinary aminoacids were normal.[4, 6]

Pathologic Anatomy

Autopsies or renal biopsies have been reported from six families.[2-4, 6-8] The anatomic diagnoses have included chronic pyelonephritis,[4, 7] chronic glomerulonephritis,[8] renal sclerosis with pyelitis,[2] and glomerulonephritis and interstitial nephritis combined[3, 6]

With the exception of a few hyalinized glomeruli and occasional foci of interstitial infiltration with leukocytes, the only abnormality found in renal biopsies of our young involved subjects was scattered foci of lipid-filled foam cells. One of these is shown in Figure 1. In the only other biopsy reported,[8] the renal histology was normal even though the patient had hematuria at the time of the examination. The initial autopsy specimen available in our family[7] showed intense interstitial pyelonephritis. Subsequent study of another patient from this family showed interstitial infiltration with lymphocytes (interstitial nephritis), glomerulonephritis, and large collections of foam cells (Figure 2), similar in all respects to those found in the biopsy specimens obtained from other patients early in the course of the disease.[8] In another family, too, the diagnosis at autopsy in two cases was pyelonephritis, but foam cells were found in addition.[4] A photomicrograph of the kidney from one of these patients is reproduced in Figure 3. Finally, another autopsy performed in a family previously stated to have glomerulonephritis[8] showed interstitial nephritis and glomerulonephritis, plus large collections of foam cells[3] (Figure 4).

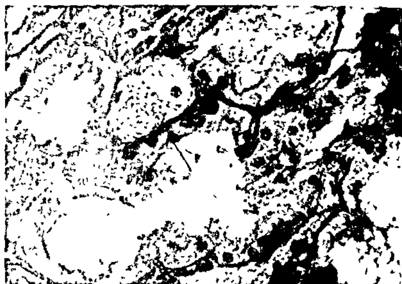


FIGURE 1.* High-power magnification of *foam cells* in a kidney biopsy

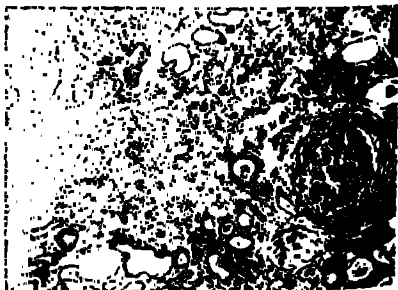


FIGURE 2. Photomicrograph of kidney at autopsy, hematoxylin-eosin stain This section shows many *foam cells*, a glomerular crescent and moderate interstitial lymphocytic infiltrate

* Figures 1, 2, and 5 are reproduced from Perkoff, G. T., Nugent, C. A., Dolowitz, D. A., Stephens, F. E., Carnes, W. H., and Tyler, F. H., *A.M.A. Arch. Int. Med.* 102:733, 1958.



FIGURE 3. Photomicrograph of kidney from Case II of second family (Reproduced from Goldbloom, R. B., Fraser, F. C., Waugh, D., Aronovitch, M., and Wiglesworth, F. W., *Pediatrics* 20 241, 1957.) Large numbers of foam cells, leukocytic infiltration and a glomerulus with fibrosis of Bowman's capsule are seen

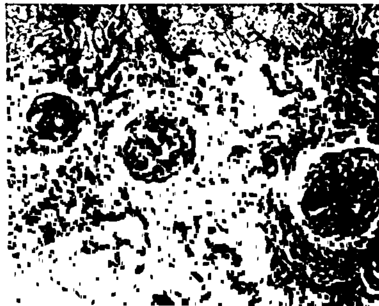


FIGURE 4 Photomicrograph of kidney from Massachusetts General Hospital Case [illegible] (*New England J. Med.* [illegible]

intermittently. Almost invariably, males are more severely involved th[illegible] females, and usually show progression of their disease to uremia and de[illegible] In each family a form of dominant inheritance is found Associated [illegible] deafness is a characteristic clinical feature of the disease in all groups [illegible] far recognized.

It was hoped that careful postmortem study of the kidneys of pa[illegible] from different families with this disease would result in clarification [illegible] the pathologic anatomy of the disorder. In this light, the variable histolo[illegible] descriptions reported in patients from different families is disturbing. T[illegible] pathologic alterations, however, do offer a clue to the pathogenesis [illegible] the disease and suggest an explanation for the variation in tissue diagno[illegible]

The *finding of foam cells in renal biopsy specimens from yo[illegible]* plus the demonstr[illegible]n of many of these cells in the kidneys at autop[illegible] in three different families with hereditary chronic nephritis, sugge[illegible] that the foam cel[illegible] represent a common anatomic denominator [illegible] the families and [illegible] reditary chronic nephritis is a disease separate fr[illegible] either typical [illegible] nephritis or glomerulonephritis. Foam cells [illegible] not a feature [illegible] histologic picture of sporadic pyelonephritis [illegible] vascular neph[illegible] *ept for their occurrence in the tubular* epitheli[illegible] some patie[illegible] the nephrotic syndrome, they do not occur regular[illegible] in glom[illegible] We have not observed the nephrotic syndr[illegible] in our [illegible] this syndrome has been reported in only one fa[illegible] with heredi[illegible] [illegible]onic nephritis.[7]

As a working [illegible]pothesis, it is suggested that patients with heredit[illegible] chronic nephritis have an abnormality of fat metabolism which lead[illegible] accumulation of fat-filled *macrophages in the kidney.* If this hypothe[illegible] is correct, the other histologic changes which have been observed [illegible] *be secondary to the* primary defect and might vary *depending* on t[illegible] degree of renal infection or the location of the groups of *foam* ce[illegible] or they might be due to other as yet unknown factors.

The relationship of hereditary chronic nephritis to sporadic pyel[illegible] phritis or glomerulonephritis is unclear. The presence of abnormal [illegible] cells in the kidney might be responsible *for an* increased incidence of [illegible] infection on a mechanical basis alone. Alternatively, the metabolic de[illegible] responsible *for lipid accumulation* might alter *the resistance* of the k[illegible] to infection, or damage glomeruli in some way. The answers to these [illegible] other questions posed by this interesting disease await *further* study

SUMMARY

(1) The clinical and genetic characteristics of hereditary [illegible] nephritis with associated *nerve deafness are* similar in several [illegible] reviewed.

(2) It is suggested that this disease is a disorder separate from classical pyelonephritis or glomerulonephritis.

(3) The foam cells found in the kidneys of some of these patients may represent anatomic evidence of the underlying metabolic disorder responsible for the renal disease.

(4) Identification of the metabolic defect in these patients should help in the understanding of the problems encountered in the study of sporadic pyelonephritis and glomerulonephritis.

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intermittently. Almost invariably, males are more severely involved than females, and usually show progression of their disease to uremia and death In each family a form of dominant inheritance is found Associated nerve deafness is a characteristic clinical feature of the disease in all groups thus far recognized.

It was hoped that careful postmortem study of the kidneys of patients from different families with this disease would result in clarification of the pathologic anatomy of the disorder. In this light, the variable histologic descriptions reported in patients from different families is disturbing. The pathologic alterations, however, do offer a clue to the pathogenesis of the disease and suggest an explanation for the variation in tissue diagnosis.

The finding of foam cells in renal biopsy specimens from young patients, plus the demonstration of many of these cells in the kidneys at autopsy in three different families with hereditary chronic nephritis, suggests that the foam cells represent a common anatomic denominator between the families and that hereditary chronic nephritis is a disease separate from either typical pyelonephritis or glomerulonephritis. Foam cells are not a feature of the histologic picture of sporadic pyelonephritis or vascular nephritis Except for their occurrence in the tubular epithelium of some patients with the nephrotic syndrome, they do not occur regularly in glomerulonephritis. We have not observed the nephrotic syndrome in our patients, and this syndrome has been reported in only one family with hereditary chronic nephritis.[7]

As a working hypothesis, it is suggested that patients with hereditary chronic nephritis have an abnormality of fat metabolism which leads to accumulation of fat-filled macrophages in the kidney. If this hypothesis is correct, the other histologic changes which have been observed might be secondary to the primary defect and might vary depending on the degree of renal infection or the location of the groups of foam cells, or they might be due to other as yet unknown factors.

The relationship of hereditary chronic nephritis to sporadic pyelonephritis or glomerulonephritis is unclear The presence of abnormal foam cells in the kidney might be responsible for an increased incidence of renal infection on a mechanical basis alone. Alternatively, the metabolic defect responsible for lipid accumulation might alter the resistance of the kidney to infection, or damage glomeruli in some way. The answers to these and other questions posed by this interesting disease await further study.

Summary

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GENERAL DISCUSSION

DR. SHAPIRO: We are pleased to hear the comment which Dr. Kimmelstiel made to the effect that it is not necessary to have vascular lesions and hypertension in order to make the diagnosis of chronic pyelonephritis. To those of us who have been struggling with the problem of the association of pyelonephritis and hypertension, the thing that has always been impressive is not that some chronic pyelonephritics have hypertension, but rather how inconsistent this phenomenon is and that so many severe pyelonephritics have no significant hypertension. It becomes somewhat difficult, therefore, to insist that the presence of hypertension or vascular disease is a necessary qualification for making a diagnosis of pyelonephritis

Experimentally, too, we have been impressed by our inability to produce significant hypertensive vascular disease and hypertension in the rat with chronic pyelonephritis in studies similar to those which Dr. Heptinstall presented this evening This applies with all three bacterial species that we have used – *Escherichia coli*, enterococci, and *Proteus* – with the exception that the rats infected with *Proteus* may show a slightly higher blood pressure than normal animals, but not to significant hypertensive levels, and unassociated with either vascular disease or increase in heart weights. The latter, as those of you who have taken rat blood pressures know, are probably even more important to the demonstration of true hypertension than are the levels of pressure themselves, which are of necessity only systolic levels

One alternative explanation for the association between chronic pyelonephritis and hypertension clinically may perhaps be related to the observation by ourselves and others that in certain types of hypertension in the rat, an increased susceptibility to pyelonephritis can be demonstrated

More recently we have been able to elicit data indicating that in animals with pre-existing DOCA hypertension the production of pyelonephritis, if this is done with an organism such as *Proteus* which produces persistent infection, can aggravate the hypertension and result in vascular changes within a period of three to four months that resemble those which Saphir and others have described as typical of pyelonephritis lenta.

This brings me to a question for the pathologists, and Dr. Kimmelstiel in particular. Have you tried to study the incidence of pyelonephritis in the kidneys of hypertensive patients in contrast to the incidence of pyelonephritis in other nonhypertensive diseases of the kidney? After all, we accept the fact that other types of renal disease predispose the kidney to the development of pyelonephritis, and hypertension is in a sense a disease of the kidney per se.

DR. KIMMELSTIEL: I thought I had answered that in part before by saying that we examined 100 cases of malignant nephrosclerosis and found pyelonephritis in 15 per cent.

DR. SHAPIRO: I mean in nonmalignant hypertension — the disease we ordinarily call essential hypertension.

DR. KIMMELSTIEL: No, I have not made this study, and I would not be able to comment on it. However, I should like to say this about it.

In the first place, from what I have read (and I have not seen the sections), I take it that in the cases of experimental hypertension with DOCA the animals are more susceptible to pyelonephritis than are normal animals. However, in these same experiments it is also noted that a tubular dilatation takes place under the circumstances. Therefore, we do not have just simply hypertensive kidneys, but we also have, apparently, kidneys in which there is some sort of stagnation of urinary flow which may add another factor to the pathogenesis of pyelonephritis.

Secondly, it strikes me as quite significant that there is a discrepancy between animal experiments and human experience. There is no doubt that in human chronic pyelonephritis vascular changes occur frequently and most conspicuously in the areas of pyelonephritis. For instance, in unilateral pyelonephritis, as I mentioned, we have had 6 cases in which the opposite kidney was not involved at all.

In animals, however, there is no involvement of vessels. So I wonder whether the answer is either that the type of pyelonephritis experimentally produced is really not the same as it is in human beings, or else we haven't waited long enough. Maybe vascular changes occur in animals at a much later date. I am not familiar with the details of the duration of these experiments.

I may make a remark on the work of Kincaid-Smith. We were struck by her idea that the vascular changes may be due to an arteritis occurring in the acute stage of pyelonephritis. We did what she did, namely, we went back to cases of acute pyelonephritis. In only one out of a large series of cases of acute pyelonephritis did we see an arteritis. The rest of the cases did not show it.

I wish to make one comment on the foam cells. Foam cells of the kidney are not as infrequent as one may think. They occur, for instance, in what is now called sclerotic glomerulosclerosis, a recently publicized renal lesion. I have seen them also in cases of diabetes, and they are well known in cases of pyelonephritis.

We refer to those things as xanthomatous pyelonephritis. I have an idea that in these cases the foam cells actually are due to an inflammatory

lesion in the pelvis or near the pelvis, where they have taken up the fat from the destroyed surrounding fat tissue.

DR. PERKOFF: In answer to Dr. Kimmelstiel's comment, the lipid in these kidneys is distributed diffusely as yellowish interstitial streaks and is quite different in appearance from that of the lipid "xanthomas" of pyelonephritis. In addition, none of these patients had liver disease or diabetes.

DR. GRIFBLE: I should like to make a few remarks in regard to the relationship between pyelonephritis and hypertension. Two of the three cases of pyelonephritic contracted kidneys described by Lohlein in 1917 were hypertensive, and subsequently many correlations have been made wherein the association was striking.

After Goldblatt's classic experiment, attention was focused (clinically at least) on unilateral renal disease, and pyelonephritis was the most frequent lesion in association with hypertension. On the other hand, the correlation between unilateral renal disease and its severity, as done in the Mayo Clinic in a large series, did not show a relation to hypertension.

In a study on 3000 autopsy cases, we could not find a correlation between the occurrence of hypertension and a discrepancy in the weights of the two kidneys. However, if the severity of pyelonephritis in both kidneys is taken into consideration, the relationship between pyelonephritis and hypertension becomes significant.

Therefore, this would appear to be a human counterpart to those animal experiments which show that functional or surgical reduction of total renal mass is productive of hypertension.

DR. SCHREINER: Dr. Kimmelstiel and Dr. Brun both gave us good reasons why there are not more published biopsy series on the pathology of pyelonephritis. Many of us are reluctant to draw statistics from such selected material, with such uncertain criteria.

Approaching our own biopsy material, which is now in the range of between 400 and 500 human biopsies, we were certain of only one thing, and that was the uncertainty of the pathologic criteria, so we undertook the following arrangement:

The pathologic slides are interpreted by ourselves on first section, independently by whatever hospital pathologist is reviewing the surgical sections, and then by access to the Armed Forces Institute of Pathology, where a separate opinion is obtained.

We are in the process of charting these separate and independent diagnoses on our entire biopsy series. However, one does not have to go

very far down the list to realize that pyelonephritis is the one disease which stands out in producing discrepancies in diagnosis, both with respect to its presence and to its relative prominence in these cases.

Perhaps this might be a good place to begin to find out what the differences are that cause some pathologists to feel strongly about certain lesions and not strongly about other lesions. It may be a mistake to exclude infarctions due to vascular involvement from pyelonephritis, as Dr. Kimmelstiel has done, although we are sympathetic with the desire to do so in autopsy material.

Certainly we have seen young children who have had the sequential relationship of bladder neck obstruction, recurrent cystitis, recurrent pyelonephritis, severe hypertension at the early age of 6, who have severe arteriolar and arterial involvement and who have these areas of infarction precisely as Dr. Kimmelstiel has shown The fact that they involve this infarction does not necessarily mean that these are not the vascular sequelae of pyelonephritis, as has been inferred by several observers

We also have several human cases of *Proteus* pyelonephritis with severe hypertension with diastolic pressures in excess of 120 mm Hg. The patients are now normotensive after prolonged antibiotic therapy

This is not the history of essential hypertension in young people, and I think we must keep open the suggestion that pyelonephritis can produce hypertension even though it may not do so in a large percentage of cases.

Lastly, there is a tendency in setting these criteria to exclude other diseases of the kidney, as Dr Brun did in cases of renal insufficiency. It is our experience that almost all cases with acute renal insufficiency that persist beyond two weeks of oliguria develop pyelonephritis by bacteriologic criteria This is a frequent complication in the late phase of acute glomerulonephritis, and membranous glomerulonephritis as seen in the nephrotic syndrome.

I hope Dr Pirani will withdraw the inference that finding pyelonephritis along with nephrosis means that pyelonephritis produced the nephrotic syndrome in this case In biopsying young nephrotics we have been surprised to find a large amount of leukocytic infiltration in these cases. One finds, if he treats and follows these patients for five or six years, that interludes of acute pyelonephritis occur, as evidenced by pyuria and bacteriuria Pyelonephritis is a frequent complication of the natural history of the nephrotic syndrome

Dr Kaye I was somewhat surprised at Dr Kimmelstiel's figure of 2 8 per cent chronic pyelonephritis in 3400 routine autopsies. Everyone here tonight is agreed on the focal nature of chronic pyelonephritis throughout most of the course of the disease I should like to ask Dr. Kimmelstiel how many blocks of kidney he sectioned, and furthermore whether he

thinks that if he sectioned the whole of both kidneys, and looked at every one of those slides, the incidence would still be 2.8 per cent.

DR. KIMMELSTIEL I don't think I can give even a reasonable estimate of the average of blocks that were taken. I can tell you that we were surprised ourselves by the findings. That we did not section both kidneys serially, I can assure you. (*Laughter*)

DR. MAXWELL: One very important thing that may come out of this meeting will be the advisability, the practicality, and the potentiality of renal biopsy in chronic pyelonephritis.

As has been pointed out, there is a great paucity of data in the literature for good reasons — one of the reasons being that the technique of percutaneous needle biopsy is not entirely without morbidity and danger, and also because it would seem unlikely that one would have a high probability of detecting this disease because of the type of lesion Therefore, because there are so few data, perhaps the people here should contribute what they have.

With regard to the possibility of macerating kidney tissue and diagnosing pyelonephritis by culturing renal tissue, we got excited about this after reading the paper by Jackson and his colleagues in the *American Journal of Medicine*, in which they reported five or six patients with sterile urine cultures in which the renal tissue grew out bacteria, and these patients were healed or cured We were struck by the fact that the majority of these patients seemed to have staphylococci growing out, and were suspicious that perhaps these were contaminants I would welcome a comment by Dr. Jackson if he is here.

We ourselves have biopsied from twenty-five to thirty patients who were strongly suspected of having chronic pyelonephritis because of persistently positive quantitative urinary cultures, or on symptomatic and clinical grounds, and out of those twenty-five to thirty patients we succeeded (after macerating the tissue from percutaneous needle biopsy) in getting a positive culture in but one patient in whom we had gotten persistently positive cultures in the urine.

Among these twenty-five to thirty patients I would say there were six to eight who had positive urine cultures with the same organism repeatedly. On that basis we have abandoned the technique However, I would ask those who use it or who advise it whether they use special culture media or whether they macerate the tissue in any particular way.

DR. JACKSON: We have not reported positive kidney cultures with negative urine cultures since bacteriuria was the starting point of our studies

Such findings, however, have been mentioned by Kark. In our studies, bacteria were recovered from the kidney in 56 per cent of cases in which the biopsy showed histologic changes compatible with pyelonephritis and 80 per cent in which there was classical interstitial nephritis. We were not always convinced that the bacteria isolated were pathogenically important even though the bacterial isolate was obtained as a single species and was present in the urine, usually in a mixed flora, in about 80 per cent of the cases with positive kidney cultures. Neither do I think they were contaminants in the usual sense of the word. In other words, I believe the organisms were in the kidney and urine but were "late-comers" or incidental to the disease process.

We also were unable to obtain positive kidney cultures from 10 patients with bacteriuria in whom the biopsy was normal as well as from 44 per cent of 25 patients with histologic pyelonephritis. Viable organisms in the kidney, therefore, are by no means a uniform concomitant of bacteriuria or even interstitial nephritis.

Regarding our biopsy experience, we expected to find (as pathologists and experience had led us to believe) that we would miss a great deal of pyelonephritis by the biopsy technique, blindly taking such a small specimen. We were surprised that 70 per cent of the patients had positive histologic findings. I should call to your attention the fact that we were studying the clinical disease, pyelonephritis, and not renal biopsy as a diagnostic technique. The patients whom we selected for biopsy had well-documented acute or chronic bacteriuria and pyuria and clinical symptoms. They were not random patients. Among the patients who had acute pyelonephritis there were no findings except the cellular and leukocyte casts in tubules and collecting ducts. The interstitial tissue was normal.

I think the strong emphasis given the focal nature of pyelonephritis is misplaced when we consider nonobstructive chronic pyelonephritis, for even though the histologic lesion is focal, the kidney is diffusely involved in 80 per cent of patients with chronic symptoms and bacteriuria as shown by biopsy results. From both clinical and biopsy observations I am not sure that acute pyelonephritis and chronic pyelonephritis are the same but progressive disease. I think there is a good deal of doubt about this.

Regarding our previously reported incidence of 9 per cent pyelonephritis in 4425 consecutive autopsies, it also was noted that pyelonephritis of major severity, perhaps conforming to the criteria of Dr. Kimmelstiel, was about 3 per cent. Recently we have reviewed the last ten years of autopsies numbering nearly 3000, and in that group we still have an 11.5 per cent incidence of pyelonephritis. In this group again one-half to two-thirds of that 11.5 per cent qualify as moderate or severe pyelonephritis.

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19

*Mechanism of Urinary Acidification**

GERHARD GIEBISCH, M.D., ERICH E. WINDHAGER, M.D.,
and ROBERT F. PITTS, M.D., Ph.D.

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The main role of the kidney in acid base balance is to regulate the concentration of bicarbonate bound cation† in the extracellular fluid. The process is a dual one: first, the salvage of filtered bicarbonate, second, the elimination of excess anions as titratable buffer acid or in combination with ammonium ions.[18, 19]

Various lines of evidence indicate that the underlying mechanisms for the renal tubular reabsorption of bicarbonate-bound cation, the acidification of urinary buffers and the exchange of urinary sodium for ammonium ions is that of an exchange of hydrogen ions formed in the renal tubular cells for sodium ions in the tubular fluid.[1, 2, 3, 4, 5] Accordingly, sodium is reabsorbed and the interaction of hydrogen ions with urinary bicarbonate leads to the formation of carbonic acid and its dissipation as carbon dioxide and water. On the other hand, conversion of alkaline buffer salts to their acid form increases titratable acidity. The establishment of a considerable hydrogen ion gradient between the cells and tubular fluid results in many species in trapping of ammonium ions.[12, 13] From a quantitative point of view the reabsorption of bicarbonate is much more important. If a hydrogen-sodium exchange is responsible for both the reabsorption of bicarbonate-bound cation and the acidification of urinary buffers, the amount of hydrogen ions secreted as titratable acid is only a fraction of that involved in bicarbonate reabsorption.

A number of uncertainties should be stressed at this point. Although many authors accept the mechanism of hydrogen-sodium exchange as fundamental for bicarbonate reabsorption and the excretion of titratable acidity, its strength lies mostly in the fact that [illegible]

* Work carried out in the laboratory of the authors was supported by a grant in aid from the American Heart Association.

† "Bicarbonate-bound cation" is a useful term in that it identifies a quantity of cation in excess of fixed nonvolatile anions. Because of the ubiquity of carbonic acid, such excess cation is always balanced by bicarbonate. The term "bicarbonate-bound cation" is equivalent to the old and to some objectionable term "base-bound base."

servations on alterations in acid-base balance, including the effects of carbonic anhydrase inhibitors,[3, 20] of changes in the carbon dioxide tension,[4, 10, 24] of changes in the potassium stores,[2, 3, 5, 27] and of the administration of nonreabsorbable anions,[14, 26] are consistent with the thesis presented. However, it should be realized that as far as the underlying mechanism is concerned, there is neither direct evidence nor rigorous proof for the existence of such an ion exchange mechanism in the renal tubule cell. Also, little is known of the nature of the proposed ion exchange process and the various driving forces involved. Thus, no rigid distinction can at present be made between carrier-linked ion exchange and a situation in which, owing to different mobility of two ion species, an electrical gradient is formed which expedites passive movement of hydrogen ions. However, a thesis taking these various aspects into account has recently been formulated [22]

It will not be the purpose of this presentation to review the mechanisms of the renal regulation of acid-base balance, since this has been the subject of a number of recent publications,[2, 10, 18, 21] but rather to discuss some recent contributions to the problem of urinary acidification In particular, experimental results obtained on single nephrons will be stressed and reference will be made to some electrical phenomena on single tubules, inasmuch as they seem pertinent to the problem of hydrogen and sodium ion exchange. Most of the observations to be described are concerned with pH changes of tubular fluid at various sites along the nephron under a variety of experimental conditions It will become obvious that these results necessitate some revision of generally held views on the site of urinary acidification in the mammalian nephron

Most theories of renal acidification hold that changes in urinary pH are exclusively the function of the distal convoluted tubule. This thesis is based on observations made on the amphibian nephron In the frog and Necturus, the injection of indicator dyes into the tubular lumen and the withdrawal of tubular fluid in micropipettes for pH measurement indicated that the urine is acidified within a limited portion of the distal tubule.[16] On recent reinvestigation, this view was fully confirmed [5] In the absence of similar direct observations in the mammalian kidney, it has been necessary to assume homology of tubular function in the amphibia and mammalia

Two lines of evidence have been cited which are contradictory to this view. In early studies on the rat kidney,[30] the tubular fluid/plasma ratios of chloride in the proximal urine were observed to be 1.4 Much evidence indicates that the fluid/plasma ratio of osmolar concentration within the same region is 1.0 If chloride is increased 40 per cent, bicarbonate must be reduced by an essentially equivalent amount. If the pCO_2 of tubular

fluid is the same as that of plasma, a reasonable assumption, then proximal urine must be highly acid. Some were not impressed by this argument, for quantitatively it is impossible. If all bicarbonate were reabsorbed, the chloride could increase no more than 25 per cent.[21] Because of this obvious discrepancy, the observations were not accorded their due qualitative significance. A second line of evidence,[17] indicating proximal acidification, was the deposition of Prussian blue in the brush border of the dog tubule following the intravenous injection of ferric ammonium citrate and sodium ferrocyanide. The reaction of these two compounds occurs only in solutions more acid than pH 5.5. There is today no evidence that proximal urine under any circumstance can be acidified to this degree. However, it is possible that the hydrogen ion concentration at a secreting surface could be considerably higher than in the depths of the luminal fluid. Again this work may not have been accorded proper qualitative significance. The problem of urinary acidification in mammalian species seemed important enough to warrant a direct micropuncture study. Such projects have been carried out independently by Gottschalk, Lassiter, and Mylle,[11, 12] by Litchfield in Bott's laboratory,[13] and by Windhager and myself.[9] The results obtained indicate that in the rat a varying degree of acidification occurs at sites other than the distal convoluted tubule.

TABLE I. MEASUREMENTS OF pH IN PROXIMAL TUBULAR FLUID DURING INFUSION OF 20% MANNITOL

Site % Proximal	pH TF	pH Blood	pH Urine	pH TF−B	pH U−B
60	6.89		6.50	-	
40	7.08	7.32	6.49	−0.24	−0.83
60	7.00	7.32	6.11	−0.32	−1.21
40	6.97	7.38	6.58	−0.41	−0.80
30	6.85	7.38	6.55	−0.53	−0.83
ADDITIONAL INFUSION OF CARBONIC ANHYDRASE					
40	6.89	7.43	6.82	−0.54	−0.61
40	7.34	7.40	6.60	−0.06	−0.80
50	6.90	7.40	6.58	−0.50	−0.82

In 3 animals carbonic anhydrase (International Biochemical Corporation) was administered, 6.1 mg was given as prime and 40 mg dissolved in 25 ml of 20% mannitol solution. The latter was infused at a speed of 0.08 ml/min.

First, I wish to discuss results obtained during osmotic diuresis. Table I gives results of a representative group of experiments which were carried out in rats during the infusion of 20 per cent mannitol at rates sufficient to lower creatinine U/P ratios below 10. Methods of micropuncture and

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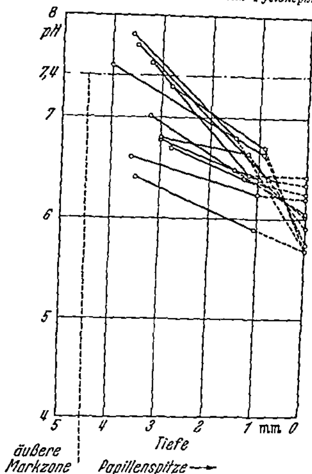


FIGURE 1 Data obtained in the golden hamster on pH of fluid collected from single collecting ducts by microcatheterization Collection of fluid and measurement of pH was carried out at a pCO_2 of 40 mm Hg pH is plotted ... al papilla Points at the right ... urine (Data from Ullrich, ... ysiol 267 401, 1958 Figure ... ark, Struktur, Stoffwechsel und Funktion," *Ergebn. Physiol.* 50 433, 1959.)

relatively negative with respect to the tubule lumen. It has been pointed out[22] *that this electrical potential difference is of such magnitude in the* proximal tubule that it probably can be overcome by the normally existing concentration gradient for hydrogen ions between cell interior and tubule fluid, thus making the existence of an active hydrogen ion transport system not an a priori necessity.[22] It is obvious that any increase in intratubular negativity reduces the electrical gradient between tubule

lumen and cell interior, thus reducing the electrical gradient which has to be overcome by the hydrogen ion movement Two such situations may be realized First, the intratubular negativity seems to be higher in more distal regions of the nephron,[6, 25, 31] a condition which would impose less of a bucking-voltage on hydrogen ion influx. Second, the presence of nonreabsorbable anions in the tubular fluid, well known to increase hydrogen ion excretion,[14, 26] was also found to increase intratubular negativity.[7] It appears from these two suggestions that the electrical gradient between cell interior and tubule lumen may be one of the factors involved in determining tubular hydrogen ion transport.

Conclusion

A review of recent studies done on single nephrons shows that in the mammalian kidney, significant acidification occurs during passage of tubule fluid, either through the proximal tubule or the collecting duct system, while the distal convolution plays a less important role While in osmotic diuresis, respiratory acidosis, and metabolic acidosis the proximal tubule seems to accomplish considerable acidification, studies done on nondiuretic animals and experiments performed in a state of isotonic sodium chloride diuresis or hypokalemic alkalosis indicate that the most significant pH changes occur at a more distal site, predominantly in the collecting ducts.

Acknowledgments

The authors are indebted to Drs Gottschalk, Lassiter, and Mylle, and to Drs. Litchfield and Bott, for permission to quote extensively from their unpublished experimental results

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In 1956[9] we had demonstrated by micropuncture in a study confirming Walker, Bott, Oliver, and MacDowell[4] which in turn has been confirmed recently by Gottschalk and Mylle[1] that in the early distal convolution of concentrating kidneys the tubule fluid is distinctly hypotonic and reaches isotonicity somewhere before entering the collecting duct. At no time could there be demonstrated a hypertonic tubule fluid at any point of the renal cortex. The final urine concentration must occur along the collecting duct.

The second round, which has already been opened, cannot be expected to be terminated in the near future. The mechanisms of transport, active and passive, the selective permeabilities, the interferences of substances other than sodium and water, are so intricate that only a tentative description of the concentrating mechanism may be given at the present time.

The active part seems to be played by a transport of sodium out of the ascending limb into the interstices of the medulla. Since in the beginning of the distal convoluted tubule the fluid is invariably hypo-osmotic, the ascending limb must be virtually impermeable to water. That sodium is the substance actively transported follows *per exclusionem* from the fact that sodium (with a concomitant anion) is the only solute available in sufficient amount to account for the hypotonicity observed. Furthermore it was shown by Gottschalk and Mylle that more pronounced hypotonicities may be found in early distal convolutions during saline diuresis than during mere hydropenia or an osmotic diuresis induced by mannitol, glucose, or urea.

The fate of this sodium is not entirely known. Apparently it is not removed efficiently from the medulla by the vasa recta, which, as discussed earlier, represent a countercurrent exchange system So the medullary interstitial space is made (slightly) hypertonic. This in itself would not suffice for the operation of the countercurrent multiplier For this an indispensable prerequisite is the entering at the hairpin bend of a (slightly) hypertonic solution in the ascending limb. This may be achieved by an osmotic equilibration of the contents of the descending limb with the hypertonic interstitium. Whether sodium is taken up actively in the descending limb or passively, requiring a selective permeability, whether a water movement accompanies the sodium transport to the descending limb or, finally, whether the equilibration is performed by water movement alone, must be left open for further investigation.

Another question which is still open concerns the extension of the sodium transport activity and water impermeability of the ascending limb. Is it the whole limb, including the thin segment of the long loops? This seems to follow from what evidence we have at hand — evidence which by necessity is all derived from postmortem examinations of kidney slices.[6, 10] This assumption implies an abrupt change of function of the

thin segment at the tip of the loop in spite of the fact that no change of the morphologic appearance is evident. Or is the activity, as Ullrich proposes, limited to the thick segment of the ascending limb? In this case the two limbs of the countercurrent multiplier would be represented by two well-defined and morphologically different structures, but the active part of the concentrating mechanism would be limited to the outer medullary zone. Both alternatives are not, at the present time, entirely satisfactory.

The final abstraction of water from the collecting ducts to form a hypertonic urine is conceived as a passive process, the epithelial cells of the collecting ducts serving as semipermeable membranes. This assumption is complicated but not seriously hampered by the findings of Hilger, Klumper, and Ullrich [8] by microcatheterization that some active ion transport processes occur in the collecting ducts.

The micropuncture studies of the distal convoluted tubule published in 1956 have thrown some light on the mechanism of action of the anti-

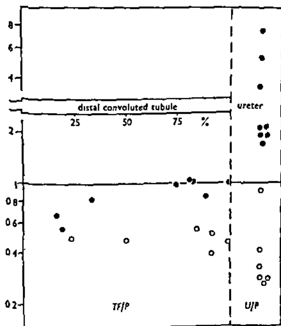


FIGURE 2. Distal tubular fluid/plasma ratios (TF/P) and ureteral urine/plasma ratios (U/P) of total molecular concentration in the concentrating (•) and the diluting (o) rat kidney. The site of micropuncture is given as per cent of the length of the distal convoluted tubule. (Reproduced from Wirz, H., *Helvet physiol. et pharmacol. acta* 14 353, 1956)

a wide range by the infusion of mannitol. It is apparent from Figure 1 that at every level of solute excretion, the urine produced during hypercalcemia was more dilute than urine excreted by the same animal under similar circumstances before injections of parathyroid extract. After

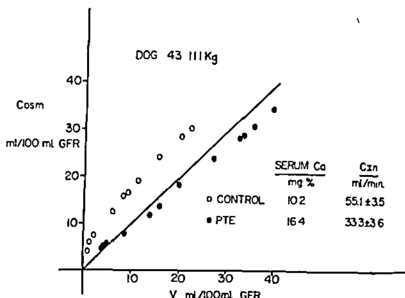


FIGURE 1 Impairment of $T^c_{H_2O}$ after treatment with parathyroid extract (PTE) The heavy diagonal line is the isotonic parameter. Points above this line represent urines more concentrated than plasma, points below the line, urines hypotonic to plasma Note that after administration of parathyroid extract (black dots) urine became progressively more hypotonic with increasing mannitol diuresis, despite a constant intravenous infusion of vasopressin

hypercalcemia was induced, in fact, the urine rapidly became more dilute than plasma when mannitol was infused, despite the simultaneous infusion of exogenous antidiuretic hormone. Similar findings were reported in potassium-deficient dogs by Giebisch and Lozano.[6]

What are the structural alterations in the kidney associated with this striking change in renal concentrating ability? On microdissection, lesions are seen which are localized to focal areas of the ascending limb of Henle's loop, the distal convoluted tubule, and most prominently, throughout the entire collecting system[1] Figures 2 and 3 illustrate typical changes The epithelial cells of the collecting ducts show swelling, calcification, and necrosis, and in some ducts there is calcification of the basement membrane as well. In contrast to the collecting ducts and distal tubules, proximal tubules show surprisingly little damage although, with more prolonged hypercalcemia, lesions appear in the proximal tubules and

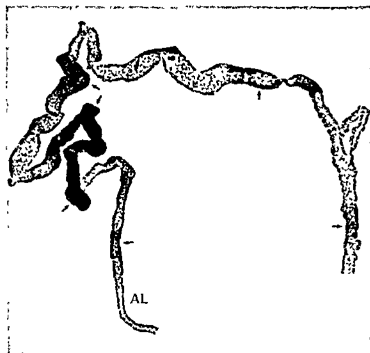


FIGURE 2 Microdissection of a cortical collecting tubule, distal convolution and ascending loop of Henle (AL), from the kidney of a dog which had been treated with parathyroid extract Fatty changes are diffuse but are more marked in the segments indicated by arrows

glomeruli as well Changes entirely similar to these are seen in the kidneys of rats intoxicated with vitamin D

One would expect that such strategically placed lesions might disrupt the countercurrent mechanism by which sodium is concentrated in the medulla and papilla of the kidney Figure 4 illustrates that this is indeed the case Normal rats and rats made hypercalcemic with vitamin D were given vasopressin, their urine was collected, and portions of their kidneys were analyzed. Both groups of animals had been placed on a sodium-free diet prior to the experiment so that the concentration of sodium in the urine would not obscure the interpretation of the analysis of kidney tissue from papilla and medulla Maximum urinary concentration was greatly depressed in the hypercalcemic animals This was associated with a decrease in the content of sodium of medulla and papilla This change is a highly significant one, expressed as milliequivalents of sodium per unit either of tissue water or of tissue solids.[8] The hypothe-

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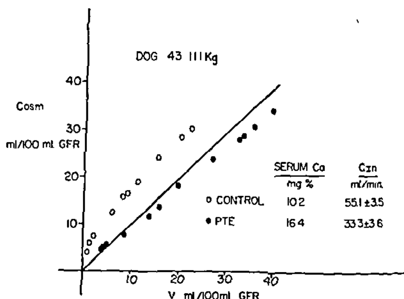


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Figure 5, which summarizes some experiments on potassium-depleted rats. (Similar results have been obtained in potassium-depleted dogs.) Like hypercalcemia, potassium depletion is also associated with a decrease in the gradient of sodium concentration from cortex to papilla. Note that the percentage fall in maximum urinary solute concentration produced by potassium deficiency or hypercalcemia is quite a bit greater than the drop in papillary or medullary sodium. This suggests that in addition to interfering with the accumulation of sodium in the interstitium of the papilla, these states may actually impair the easy diffusion of water from the lumen of the collecting ducts into the hypertonic interstitial fluid of the inner medulla and papilla.[10]

It is of considerable interest that patients and animals with potassium deficiency may dilute the urine normally in response to a water load, even though their concentrating ability is impaired.[8] In the group of rats illustrated in Figure 6, *maximum* urinary concentration (reached after dehydration and vasopressin) was clearly diminished by potassium depletion. Nevertheless, the *minimum* osmolality of the urine achieved during water diuresis was not significantly changed in the potassium-deficient state. Since urine is presumably diluted by the reabsorption of

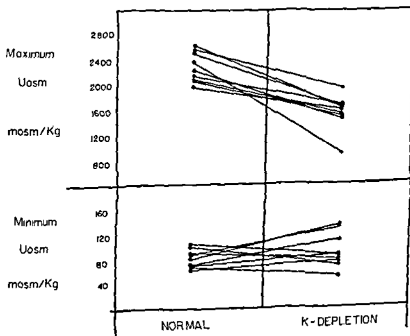


FIGURE 6 Potassium depletion decreased renal *concentrating* ability (top) but did not significantly affect *diluting* capacity (bottom)

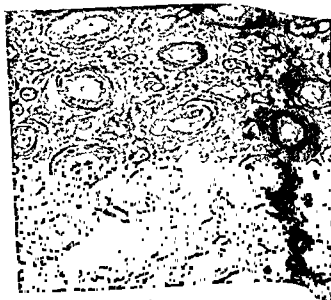
sodium without water in the loop of Henle,[11] this experiment suggests that active reabsorption of sodium in Henle's loop is not appreciably affected by

Gottschalk

of potassiur

ducts of structural changes seen by the morphologist.

The importance of the collecting ducts in the renal concentrating process is illustrated by the interesting morphologic findings in the following patient who had an unusual variety of nephrogenic diabetes insipidus.[1] This was a 37-year-old man who had had chronic osteomyelitis for 23 years. For several years he had noted moderate polyuria and polydipsia. The daily output of urine varied from 3 to 5 liters. The blood nonprotein nitrogen was normal and PSP excretion was 40 per cent in 2 hours. To rule out posterior pituitary insufficiency, a Hickey-Hare test was performed. Urine flow was initially 10 cc. per minute, and the concentration of the urine (about 200 mOsm per liter) was below that of plasma. Infusion of hypertonic saline did not increase the concentration of the urine,



although urine flow declined slightly. Subsequent injection of Pitressin similarly did not affect the concentration of the urine and did not result in antidiuresis. The patient died 4 months later as a result of carcinoma of the lung and multiple pulmonary emboli. At post mortem a peculiar



FIGURE 8 Microdissection of a continuous collecting duct arborization. The basement membrane of segments A and B *from the medulla* is markedly thickened by amyloid. In segment C, *from the corticomedullary junction*, there is minimal patchy amyloid deposition below but the remainder of the tubule is unremarkable

kind of amyloid infiltration was found to involve both kidneys. Unlike the usual case of amyloid disease, in which glomeruli and blood vessels are chiefly involved, the deposition of amyloid in these kidneys was predominantly in the medulla, in cuffs surrounding renal tubules (Figure 7). Microdissection revealed that the deposits of amyloid were almost entirely localized to the walls of the collecting ducts (Figure 8). Note that the amyloid deposits are thickest in the inner medulla and become less marked as the cortex is approached

It seems likely that in this case polyuria and hyposthenuria were the results of the peculiar pathologic changes in the collecting ducts. It is tempting to speculate that the cuffs of amyloid interfered with the back diffusion of water from the lumen of the collecting tubules to the interstitium of the medulla (They may also, of course, have impaired the active reabsorption of sodium by collecting duct epithelium) In any case, these findings emphasize the role of the collecting ducts in the formation of a concentrated urine in man

Summary

The countercurrent hypothesis has provided an exciting opportunity to correlate renal structure and function in normal and pathologic states It would appear that diseases of the medulla of the kidney and especially of the collecting ducts may be expected to result in early and striking disturbances in the ability of the kidneys to concentrate the urine, which are likely to be disproportionate to the degree of impairment of other renal functions In some cases, hyposthenuria reflects a decrease in the concentration of sodium in interstitial fluids of the medulla and papilla In addition, it is possible that injury to the lining of the collecting ducts may under certain circumstances interfere with the passive reabsorption of water from these structures which normally occurs under the influence of vasopressin

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GENERAL DISCUSSION

DR WELT· Dr. Gottschalk, you have been involved rather liberally in the first three papers. I wonder whether you would be willing to start the discussion.

DR. GOTTSCHALK· I have only one very brief comment to make at this time, and it is only to reinforce what Dr. Darmady said yesterday. If I misinterpreted his statement I hope he will correct me.

We can readily observe in the living hamster papilla that there are differences in the morphology of thin descending as compared with thin ascending limbs. The lumen of the thin ascending limb is wider than the lumen of the thin descending limb, and the same differences can be seen as far as the exterior diameter of the microdissected loop of Henle. We have seen these differences both in the hamster and in the rat kidney.

These differences are quite real Frankly, I am surprised that they have not been described. I would emphasize that the differences in diameter of the thin ascending as compared with the thin descending limbs are not nearly as prominent as that between thin ascending and thick ascending limbs

DR DARMADY· I should like to congratulate Dr. Epstein on his paper, which I found most interesting.

There are two problems which worry me a bit because in our experiments with vitamin D, using radioactive calcium-45, we found that there was an increased amount of calcium in the proximal tubules. Whereas I agree entirely with the mechanism Dr. Epstein has suggested, I am concerned about the first changes that occur in the proximal tubules.

When we worked with potassium-depleted rats we found that they became Pitressin-resistant within 24 hours of the start of the experiment, although from the morphologic point of view we could not see anything wrong with the tubules I wonder whether he has been able to do any analysis on the tubules for calcium or other mineral contents of the tubules.

DR. EPSTEIN. We have analyzed the cortex and medulla in a few dogs treated with parathyroid extract and in a few rats treated with vitamin D. I can speak with assurance only about the dogs treated with parathyroid extract In those dogs there was a great increase in the calcium content of the medulla, but no significant increase in the calcium content of the cortex

I am fairly certain that there is not a big increase in the calcium content of the cortex of rats treated with 200,000 units of vitamin D a day for

Dr Oliver Of course this same problem comes up in the potassium-depleted animal, because in it the intercalated cells proliferate in tremendous numbers. But the cells cut off very sharply at the junction of the outer and inner zones

I would suggest that we may be getting into difficulties of definition when we use some other methods to designate these intercalated cells than their structural characteristics; enzyme content is a functional characteristic and may be shared by other cells or tissues.

Dr Maxwell I have carried on correspondence with Dr. Oliver for ten years In 1949 I was interested in the so-called Trueta shunt, and it became important at that time to try to estimate in man the number of glomeruli in the kidney which actually had loops of Henle descending into the medulla. I conferred with Dr Oliver in Brooklyn, and we went to the literature To the best of our ability we estimated that in man at most 15 or 20 per cent of the glomeruli had loops of Henle which descended into the medulla, and that the majority of glomeruli had rudimentary thin limbs or none at all, or were confined to the cortex.

I should like to ask Dr. Oliver whether there is more information on this point, and, following him, perhaps one of the countercurrent hypothesis people would indicate whether this would change their concepts at all of how the urine is concentrated.

Dr Oliver: The answer is no, there is no more knowledge, such matters still remain a guess. We did start to make an estimate; and although we
no definitive figures, I am sure that if one could measure every nephron
y would be every length, from those that do not have any loop at
those which have long loops, the thin portion of which was both
ascending. In other words, there would probably be a
uency distribution of length
would come, and what the dispersion might be, no
point I was trying to make. Until we know
clusions can be drawn.

countercurrent thesis has two aspects
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down to the papilla, it would
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impression since the intercalated cells were intensely stained by iron hematoxylin because of their mitochondrial content. In this regard they closely resemble the epithelium of the distal convolution, and if one examines a dissection in which the continuity of the distal convolution and the collecting is preserved, one can see that the dark mitochondrial rich epithelial cells of the former have been scattered or displaced along the clear epithelium of the collecting tubule. This is the point at which the two separately developing tubular systems, nephrons and collecting ducts, meet, so dislocation of cells from one to the other might be expected I might add that these observations with the most primitive of structural techniques, microdissection, have been confirmed by Rhodin with the most elaborate, electron microscopy. The point is that the two systems, nephronic and collecting, are not so sharply separated as one is sometimes led to believe, and that the cells of the nephron spill over, as it were, into the collecting system.

But to return to my main point. We must in all temptations toward functional-structural correlations realize that to be meaningful the structural data must be as statistically valid as the functional. To the present that has not been the case, so that many so-called "correlations" are not only meaningless but at times misleading.

DR. WIRZ: Dr. Oliver, in the experiments of Ullrich and co-workers, wherein they microcatheterized collecting ducts, they found they could thread catheters up to the junction of the outer and inner zone, so that they were only getting differences in urine composition in the inner zone of the collecting ducts.

They found some signs of activity. This was done, of course, in the hamster, and I wonder if in the hamster too the intercalated cells are restricted to the outer zone.

DR. OLIVER: That is a very pertinent question. I don't know. I haven't dissected the hamster's kidney.

DR NOVIKOFF Since functional possibilities are being suggested for the distribution of intercalated cells, I should note that the tetrazolium technique (with DPNH as substrate and "nitro-BT" as acceptor) reveals their presence in the collecting ducts within the basal half of the papilla. Only in the apical half does the papilla lack such cells Perhaps the difference between Dr. Oliver's observations and ours lies in the different sensitivity of the methods used. Our method, which depends upon enzyme activity in frozen sections, may reveal mitochondria-rich cells in the collecting ducts more readily.

in 67 per cent of the animals (Figure 1). In only two of them with a particularly severe inflammation did involvement appear also in the kidney. If followed for another five to six months after the acute pyelitis, 100 per cent of the animals developed chronic interstitial renal changes spreading from the fornices into the parenchyma (Figure 2). Thus a hematogenous infection, when combined with a partial interference with free urine flow, can produce an acute pyelitis and acute and chronic pyelonephritis.

Doubts have been expressed in the past as to whether chronic interstitial inflammation of the kidney without obvious pathology of the



FIGURE 2 Chronic interstitial renal inflammatory changes in a rabbit with a partially constricted ureter, in whom several months previously an acute pyelitis had been induced by an intravenous injection of *Escherichia coli*

renal pelvis and urinary tracts is the same nosological entity, or whether its pathogenesis and pathologic basis are different Far from denying a possible different genesis for some cases, we think that the majority of these cases of interstitial nephritis, ending in contracted kidneys, belong to the same category of disease. The following arguments speak in favor of this view.

(1) In all our patients in whom we eventually obtained a specimen of kidney for examination, submucosal inflammatory infiltrates in the wall of the renal pelvis were found, even if there was no obvious present or past evidence of urological disease.

especially in those cases where free urine flow interferes with the ascent of microorganisms.

Some new light on this question has been shed by experiments on rabbits with partial narrowing of one ureter.[12] An intravenous injection of *Escherichia coli* produced an acute pyelitis on the experimental side



FIGURE 1. Acute pyelitis in a rabbit with a partially constricted ureter, [illegible] by an intravenous injection of *Escherichia coli*.

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FIGURE 1. Acute pyelitis in a rabbit with a partially constricted ureter, induced by an intravenous injection of *Escherichia coli*.

in 67 per cent of the animals (Figure 1). In only two of them with a particularly severe inflammation did involvement appear also in the kidney. If followed for another five to six months after the acute pyelitis, 100 per cent of the animals developed chronic interstitial renal changes spreading from the fornices into the parenchyma (Figure 2). Thus a hematogenous infection, when combined with a partial interference with free urine flow, can produce an acute pyelitis and acute and chronic pyelonephritis.

Doubts have been expressed in the past as to whether chronic interstitial inflammation of the kidney without obvious pathology of the



FIGURE 2 Chronic interstitial renal inflammatory changes in a rabbit with a partially constricted ureter, in whom several months previously an acute pyelitis had been induced by an intravenous injection of *Escherichia coli*

renal pelvis and urinary tracts is the same nosological entity, or whether its pathogenesis and pathologic basis are different. Far from denying a possible different genesis for some cases, we think that the majority of these cases of interstitial nephritis, ending in contracted kidneys, belong to the same category of disease. The following arguments speak in favor of this view.

(1) In all our patients in whom we eventually obtained a specimen of kidney for examination, submucosal inflammatory infiltrates in the wall of the renal pelvis were found, even if there was no obvious present or past evidence of urological disease.

especially in those cases where free urine flow interferes with the ascent of microorganisms.

Some new light on this question has been shed by experiments on rabbits with partial narrowing of one ureter.[12] An intravenous injection of *Escherichia coli* produced an acute pyelitis on the experimental side



FIGURE 1. Acute pyelitis in a rabbit with a partially constricted ureter, induced by an intravenous injection of *Escherichia coli*.

which later, for reasons so far unknown, becomes sterile and passes into a hydronephrosis The above combination of *temporary* ligature and hematogenous infection has as an immediate consequence an acute pyogenic pyelonephritis. However, some five to six months later the affected kidney becomes contracted and histologically is the seat of a typical diffuse chronic pyelonephritis, while the ureter and renal pelvis are macroscopically intact and histologically only a few submucosal inflammatory infiltrates or no abnormality at all may be found[13-15] (Figures 3 and 4) A similar result was obtained by Heptinstall and

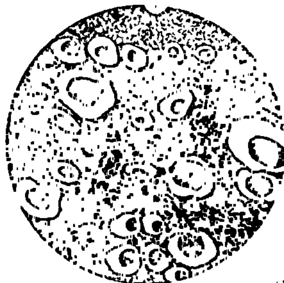


FIGURE 4 Typical chronic pyelonephritic changes (thyroid-like picture and interstitial infiltration and scarring) in a contracted kidney of a rabbit, produced as described in Figure 3

Gorrill[16] and Breinerd and Cecil[8] The morphologic analogy with human disease is good and the experiment possibly gives a clue to the pathogenesis every apparent or silent passage of a stone or some other temporary interference with free urine flow, if combined with a hematogenous infection, might start the disease, which, in terms of the pathway by which the microorganisms reach the renal pelvis, is "descending," while its spread to the kidney is "ascending" Since both etiological factors occur frequently, it is not surprising to find chronic pyelonephritis leading the list of renal diseases.

(2) On x-ray the same evidence of disturbed function of calices, pelvis, and ureter was found as in cases of primary urological disease, as will be presented later.

(3) We have succeeded in reproducing the condition in rabbits, in whom the intravenous injection of *E. coli* was effected 12 hours after complete occlusion of the ureter, which was, however, released 36 hours later. Temporary occlusion of the ureter without infection produced only a transient widening of the tubules, which often disappeared completely a few days after the release of the ligature, leaving the kidney intact Injection of *E. coli* without interference with the ureter produces practically no abnormalities of the kidney. Permanent occlusion plus infection produces acute pyelonephritis, leading to pyonephrosis



... left kidney in a rabbit ... *coli* were ... was tem-

the most frequent finding (25 p[illegible] [illegible] Escherichia coli occurring in 18.7 per cent, and *Staphylococcu*[illegible] [illegible] 14.5 per cent. The presence of organisms in the urine is there[illegible] [illegible]ite lead toward the diagnosis of an inflammatory disease affect[illegible] [illegible]inary tract and the kidney. Where bacteriologic help is avail[illegible] [illegible]antitative bacteriologic exam-

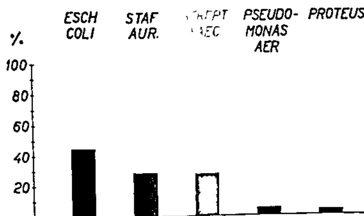


FIGURE 8 Incidence of individual microbes in urine in chronic pyelonephritis

ination of the urine (analogous to the Addis count) might be attempted. It was found by Červinka [8] that in healthy females urine practically never contained more than 10 organisms per milliliter of urine, while in healthy males counts up to 1000 per milliliter were occasionally found, due obviously to the above-mentioned contamination from the navicular fossa (Figure 9). In 40 per cent of subjects with other evidence of chronic pyelonephritis, counts over 10,000 per milliliter were found. The high incidence of zero counts in chronic pyelonephritis was, no doubt, due to intensive antibiotic and other therapy

In addition to microorganisms evidence of inflammatory process in the close proximity to the urinary passages may be found in the urinary deposit, especially if examined quantitatively. Leukocytes in the urine are the prevalent element in 75 per cent of cases, while a similar finding was encountered only in 14 per cent of glomerulonephritics and subjects with vascular nephrosclerosis (Figure 10) An absolute prevalence of erythrc cytes, frequent in glomerulonephritis (40 per cent), occurred in onl 16 per cent of cases of chronic pyelonephritis (mainly secondary t nephrolithiasis or some other primary urological disease). A parall marked increase in erythrocytes and leukocytes in the urine was found i one-third of glomerulonephritis cases and weighs strongly against chroni

Pathogenetic microorganisms are present in the urine of 81 per cent of patients (Figure 7) (*Staphylococcus albus was not thought to be* pathogenic unless plasma coagulase-positive.) In healthy subjects microorganisms might be cultivated from the urine in 6 per cent of instances They were not found in a single female where urine was sampled by a

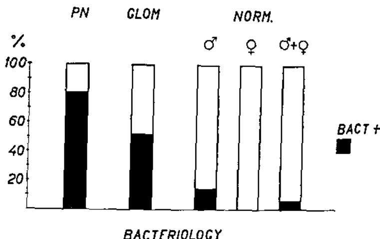


FIGURE 7 Frequency of positive bacteriologic cultures in urines.

bladder catheter, while 14 per cent of male urines, voided as a midstream specimen, gave a positive culture. This difference in incidence is probably due to the different technique of obtaining the specimen for culture and speaks in favor of the view of Kočvara and Červinka [11] that the organisms in male urine originate in the navicular fossa, from which they were able to obtain positive cultures in 100 per cent of healthy subjects This view is further strengthened by the fact that the incidence of positive cultures was reduced to zero in 31 out of 32 subjects by swabbing the navicular fossa with a disinfectant prior to micturition. Urines of patients with glomerulonephritis gave positive cultures in 52 per cent of cases and we have no explanation to offer for this finding Of the pathogenetic organisms in chronic pyelonephritis, *E. coli* headed the list with an incidence of 45 7 per cent, but was never found in the urine of healthy subjects. Second on the list was *Staphylococcus aureus* (28 6 per cent), followed by *Streptococcus faecalis* (27 per cent) On the other hand *Proteus* and *Pseudomonas aeroginosa*, frequently found in heavily infected urological cases, occurred in our material in only 3 to 4 per cent (Figure 8). Frequently two and even three varieties of organisms may be found simultaneously or in succession The distribution of various organisms in glomerulonephritis is different *Streptococcus faecalis* being

where no such reasons were present, the difference was 53 5 per cent. There is very little overlapping between subjects with symmetrical renal disease and chronic pyelonephritis Thus this simple method affords a very valuable diagnostic clue.

Of the other tubular functions, the ability to cope with an acid load was investigated by Dr Fencl in our institute after oral administration of 5 Gm of ammonium chloride per 24 hours for 2 days. It was found that during the last 12-hour period of the test, subjects with normal kidneys conserved over 90 mEq. of base in exchange for hydrogen and ammonia ions (Figure 16) This ability was impaired even in subjects with mild chronic pyelonephritis (maximum concentrating ability still above 1.020) and it dropped to about 50 per cent of its normal value in severe cases of chronic pyelonephritis. The disability is due in the first place to an impaired production of ammonia. The hydrogen exchange increases from 11 to 17 per cent of the total base-saving mechanism, but in view of its obvious limitations this mechanism per se cannot cope with the excess acid load. This agrees well with the older work of Henderson and Palmer[4] in men with gravely diseased kidneys, and with the findings of others.

A natural consequence of this impairment of excretion of hydrogen ions is a state of chronic acidosis Its close correlation with the reduction of maximum concentrating power is clearly visible from Figure 17 The reduced ability to exchange sodium for hydrogen ions leads to an enhanced loss of sodium from the body with a permanent tendency toward dehydration. Retention of fluid with edema does not belong to the functional and clinical picture of the disease. The chronic acidosis of pyelonephritis, however, only very rarely produces disturbances of calcium metabolism and skeletal changes, described by Albright *et al.*[1] in cases of "tubular insufficiency without glomerular insufficiency." The probable explanation may be found in Figure 18 correlating alkaline reserve with the rate of glomerular filtration. The degree of correlation is only slightly less than with the maximum concentrating power, suggesting that by the time the ability to produce ammonia has been severely restricted, the disease process has also severely encroached upon the glomerular surface. A retention of phosphate, which results, acts against the development of osteomalacia. It might favor, of course, secondary hyperparathyroidism, but in the last five years, since our attention has been particularly focused in this direction, no clinically recognizable case has been encountered.

X-ray investigation has also added valuable diagnostic and differentia[illegible]ta, as worked out on serial urographs and pyelographs (Figure [illegible]) Attention should be called to some striking features, such as change[illegible] kidney size and density of the kidney shadow, and [illegible] ma, which occur in 76 3 per cent of cases of chronic

process might be discovered by studying the U/P ratios, or simply the concentrations of endogenous creatinine in urine, collected by ureteral catheters separately from both kidneys.[12] This assumption proved to be correct. The difference in U/P ratios in subjects with healthy kidneys amounts to 6.0 per cent, and in subjects with renal diseases affecting both kidneys symmetrically (glomerulonephritis, vascular nephrosclerosis) the differences were similar (Figure 15). On the other hand, in subjects where chronic pyelonephritis was diagnosed on clinical or functional grounds, the difference amounted to 78 3 per cent. In those among the latter, where other reasons led us to suspect unilateral disease, the average difference for the whole group ran as high as 123,6 per cent. However, even

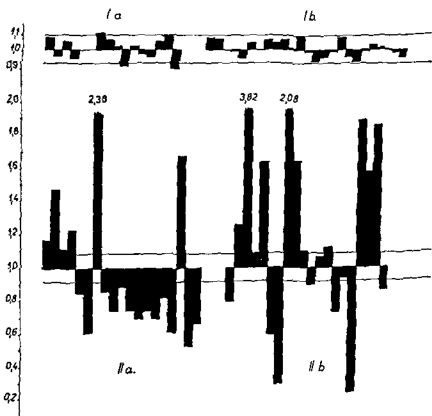


FIGURE 15. Ratio of left/right concentrating indices of endogenous creatinine ... collected separately from both kidneys by ureteral catheters Ia, ... sclerosis, ... re- ... c) ... rd of a unilateral disease The thin horizontals ... deviations from the mean.

where no such reasons were present, the difference was 53.5 per cent. There is very little overlapping between subjects with symmetrical renal disease and chronic pyelonephritis Thus this simple method affords a very valuable diagnostic clue.

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X-ray investigation has also added valuable diagnostic and differential data, as worked out on serial urographs and pyelographs (Figure 19) Attention should be called to some striking features, such as change in kidney size and density of the kidney shadow, and width of parenchyma, which occur in 76.3 per cent of cases of chronic pyelonephritis,

as contrasted with 5.6 per cent in chronic glomerulonephritics used as controls.

Further, there are typical deformations of the renal passages in the region of the papilla in chronic pyelonephritis, that is, flattening to disappearance of the papilla and of the fornix, found in 95.9 per cent of cases of chronic pyelonephritis and not at all in our group of glomerulo-

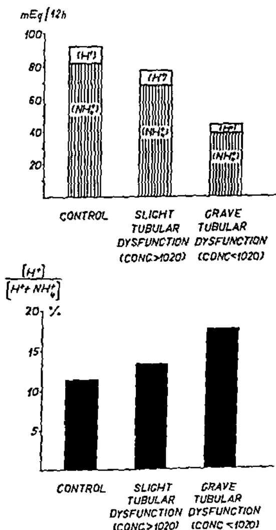


FIGURE 16 Rate of excretion of H^+ and NH_4^+ ions during the last 12-hour period of an NH_4Cl load, administered in 5 Gm doses per 24 hours for 2 days

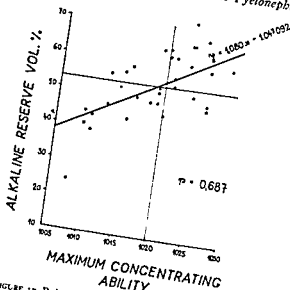


FIGURE 17 Relationship of alkaline reserve to maximum concentrating ability in subjects with chronic pyelonephritis If arbitrary lines are drawn at maximum specific gravity of 1.020 and at alkaline reserve of 52 volumes per cent, only 4 subjects who concentrate above 1.020 have an alkaline reserve below 52 volumes per cent and only 4 subjects with a maximum concentrating ability of less than 1.020 have an alkaline reserve above 52 volumes per cent

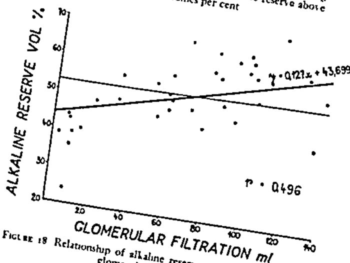


FIGURE 18 Relationship of alkaline reserve to the average 24-hour glomerular filtration rate.

nephritics There is a frequent occurrence of dilatation of the urinary passages, with functional disturbances in the dynamics of urine transport – that is, hypotonic and hypodynamic changes. This latter sign was shown in 68.6 per cent of pyelonephritics, in 1.8 per cent of glomerulonephritics.

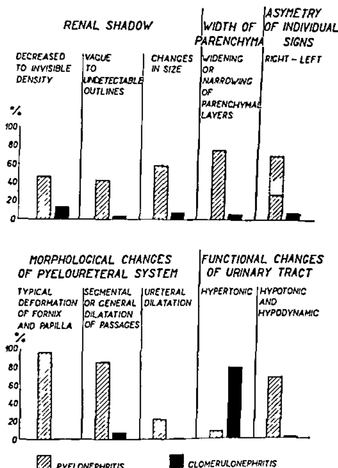


FIGURE 19 Summary of x-ray findings on intravenous pyelography (occasionally confirmed by ascending pyelography) in 153 subjects with chronic pyelonephritis and 53 with chronic glomerulonephritis

A sign of great importance is left-right asymmetry in the development of these changes. Such asymmetry varies between 27.5 and 70.5 per cent of cases. In chronic glomerulonephritis such asymmetry does not exceed 7.4 per cent.

These x-ray changes have the same incidence in primary and secondary forms of pyelonephritis

It would appear from a detailed analysis of these data that the usual x-ray techniques of excretion urography and pyelography are indispensable for complete evaluation of the picture of the chronic pyelonephritic kidney.[7, 8]

Thus, from the functional point of view, chronic pyelonephritis differs markedly from other frequent renal diseases. The functional differences appear early in the course of the disease and are present even in its most advanced stages Their recognition is within the possibilities of even small hospital laboratories, equipped with a microscope, a small centrifuge, a cell counting chamber, a urometer, and a photoelectric colorimeter for the estimation of creatinine The methods are not time-consuming and have been introduced today in over 50 hospitals in Czechoslovakia The improved early diagnosis which resulted has dispelled much of our previous therapeutic gloom.

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Prat, V, Benešova, D, and Červinka, F Experimentalni pyelonefritis III

Results

The functions of the separate kidneys were essentially the same in the preinduction studies.* Subsequent to the induction of unilateral pyelonephritis, absolute values for glomerular filtration rate (GFR), PAH clearance (C_{PAH}), maximum tubular reabsorption of glucose (Tm_g), and maximum tubular secretion of PAH (Tm_{PAH}), were decreased in the diseased kidney relative to the contralateral normal organ. Values for the diseased and normal kidneys of two representative dogs are shown in Figure 2.

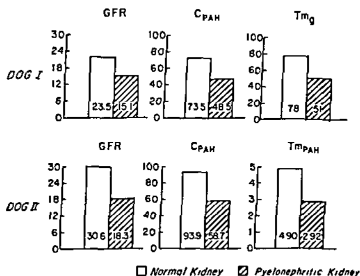


FIGURE 2 Clearance values for two representative dogs with unilateral pyelonephritis. The crosshatched bars refer to diseased kidneys, the open bars to contralateral normal kidneys Definition of symbols GFR, glomerular filtration rate (ml./min.), C_{PAH}, para-aminohippurate clearance (ml./min.), Tm_g, maximum rate of tubular reabsorption of glucose (mg./min.), and Tm_{PAH}, maximum tubular secretion of para-aminohippurate (mg./min.).

Of considerable interest is the fact that each of the various functional parameters was decreased to approximately the same degree. When the clearance ratios of the pyelonephritic kidney are compared with those of

* When significant differences existed between the right and left kidneys, the animals were not used for further study.

the contralateral control kidneys no major differences are found (Figu 3). Filtration fractions are essentially the same, GFR/Tm_g ratios a essentially the same and so, also, are the GFR/Tm_{PAH} ratios The fact th filtration fractions in the diseased kidney are comparable to those in th normal organ implies that per unit of renal plasma flow, the same volum of glomerular filtrate is formed in the functioning nephrons of the diseased

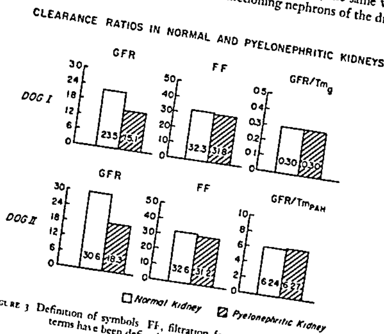


FIGURE 3 Definition of symbols FF, filtration fractions (per cent), other terms have been defined in the legend of Figure 2

organ as in those of the control kidney. Equality of GFR/Tm ratios suggests that per unit of glomerular filtrate the maximal capacity to transport glucose and/or PAH is the same in the nephrons of the diseased kidney as in those of the normal organ. These data thus imply that the balance between glomerular and tubular functions in the surviving nephrons in the pyelonephritic kidney is comparable to that found in the normal kidney It is difficult to reconcile this with the thesis that the residual nephrons of the pyelonephritic kidney include a sizable population of units in which function has been disturbed by random and chaotic morphologic alterations

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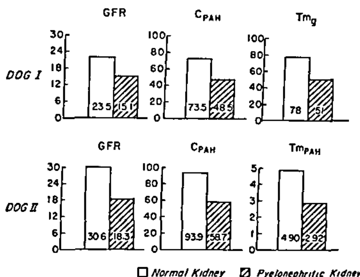


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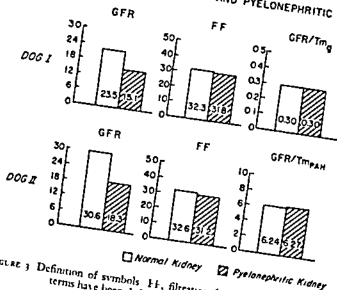


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To investigate the degree of homogeneity of the functioning nephrons of the diseased kidney more precisely, glucose titration curves were performed.[11] This technique consists of the progressive and stepwise elevation

of plasma glucose concentrations from normal levels to values in the vicinity of 1000 mg. per cent. In the normal dog kidney, essentially all of the filtered glucose is reabsorbed until the Tm is reached.[16] Thereafter, reabsorption remains constant and all filtered glucose in excess of tubular reabsorption is excreted quantitatively into the urine. The absence of a significant titration splay in the normal dog kidney has led to the conviction that the relationship between glomerular function and glucose reabsorptive capacity in the attached proximal tubules is essentially the same in all constituent nephrons.[18] A morphologic confirmation of this thesis has recently been obtained in studies by Bradley and associates[2] and Oliver.[13]

By comparison of the glucose titration curve of the pyelonephritic kidney with that simultaneously obtained for a contralateral normal kidney, a means is provided for the investigation of the homogeneity of the nephron population of the diseased organ[5] If the pyelonephritic kidney includes nephrons with normal glomeruli but impaired tubules ("atubular glomeruli"), filtered glucose should be excreted at relatively low plasma concentrations. Conversely, nephrons with small or partially fibrosed glomeruli but normal tubules ("aglomerular tubules") should continue to reabsorb glucose until extremely high plasma levels obtain. Both forms of abnormal nephrons could be detected if each existed in a considerable number.

Figure 4 depicts the results of six glucose titration studies on four animals with unilateral pyelonephritis. The pattern of glucose reabsorption is the same for the diseased as for the normal kidneys. Thus reabsorption is essentially complete bilaterally until the Tm is reached, and thereafter no increment in reabsorption occurs despite the elevation of plasma glucose levels to extremely high values. Comparison of the frequency distribution curves of group data from pyelonephritic kidneys with simultaneous data from normal kidneys reveals no statistically significant differences. Within the limits of accuracy of the glucose titration technique, therefore, the functioning nephrons in the diseased kidneys may be assumed to constitute a population as homogeneous as that in the normal dog kidney.

In view of the foregoing evidence that the residual functioning nephrons of the pyelonephritic kidney retain normal relationship between glomerular and certain tubular functions, and have the characteristics of a homogeneous population, it is of interest to examine several functional parameters that are known to deviate from normal in the presence of bilateral renal disease and uremia. Sodium excretion by the diseased kidney in bilateral pyelonephritis differs from that of the normal kidney in at last one major respect there is an obligatory sodium excretion of variable magnitude which continues despite salt deprivation,

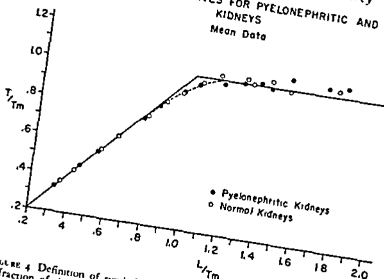


FIGURE 4 Definition of symbols T/Tm, reabsorption of glucose expressed as a fraction of the tubular maximum, L/Tm, filtered load of glucose (GFR × plasma glucose concentration) expressed as a fraction of tubular maximum

and a tendency to sodium depletion [12] Traditionally, this inability to reabsorb all the filtered sodium has been attributed to structural damage to the persisting tubules That this explanation does not apply to the pyelonephritic kidneys in the animals we studied is suggested by the data shown in Figure 5 Observations are presented for the diseased and normal kidneys of a representative animal with unilateral pyelonephritis. The animal had been fasted for 12 hours, but prior to this had received a normal sodium-containing diet The diseased kidney demonstrated the capacity to reabsorb over 99 per cent of the filtered sodium It will be noted, however, that the urinary sodium concentration was slightly higher in the urine of the diseased kidney and that the percentage of filtered sodium excreted was slightly greater These differences will be commented upon subsequently

A second functional limitation which occurs in bilateral pyelonephritis is a diminishing ability to concentrate and dilute the urine.[8, 13] The conventional explanation for these changes is that the disease process alters the functional integrity of the active sites in the residual nephrons. Experimental evidence has recently been obtained in animals with unilateral pyelonephritis which suggests that the concentrating and diluting mechanisms continue to function effectively.[4] In Figure 6a, data are shown

the functional alterations that occur in bilateral renal disease. In Figure 7*b*, the effects of mannitol and vasopressin infusions are shown in an animal with one normal and one hemi-infarcted kidney. The hemi-infarcted organ was contracted owing to the loss of a significant portion of its original

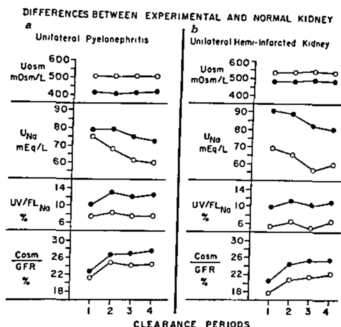


FIGURE 7 *a* The effects of mannitol and vasopressin infusion in an animal with unilateral pyelonephritis Definition of symbols U_{osm}, urinary osmolality, C_{osm}/GFR, percentage of filtered solute excreted, other terms are defined in previous figures *b*. The same experiment performed in an animal with a unilateral hemi-infarcted kidney.

nephron population (GFR was decreased by 60 per cent), but the residual nephrons were free of a progressive renal lesion, and appeared normal histologically The recorded data demonstrate differences between the *hemi-infarcted kidney* and the contralateral normal kidney similar to those that have been found between *pyelonephritic* and normal kidneys It may thus be concluded that the differences need not relate to anatomic abnormalities of the residual functioning nephrons but may be a function of adaptive changes in intact nephrons.

In an effort to explore the possibility that the differences between the pyelonephritic and contralateral normal kidneys are functional in nature, attempts have been made to modify them experimentally. A representative

experiment is shown in Figure 8 for an animal undergoing mannitol diuresis. During five conventional clearance periods, the characteristic differences emerged. Thereafter, the filtration rate of the diseased kidney

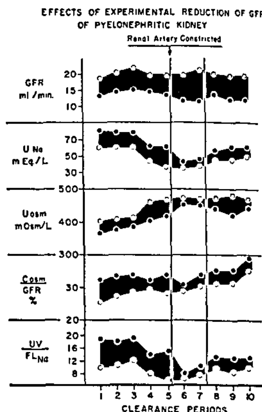


FIGURE 8 The effects of experimental reduction of filtration rate on the diseased kidney of an animal with [illegible] about the artery before the beginning of the experiment.

was diminished by partially constricting its renal artery with an arterial clamp placed about the vessel in advance of the experiment. In association with unilateral arterial compression, values for the pyelonephritic kidney approached those for the normal organ for all parameters measured.

COMMENTS

On the basis of the foregoing observations, the functional capacity of the pyelonephritic kidney in the dog may be described in terms of a diminished population of nephrons, the great majority of which are capable of normal function. No evidence has been found in support of the thesis that the pathologic alterations of specific sites in functioning units inflict diffuse and disordered functional lesions. On the contrary, all the functions studied * have been characterized by their orderly and reproducible characteristics and in most instances by their high degree of excellence.

It therefore may be suggested that partially disabled nephrons did not contribute in a decisive manner to the over-all function of the pyelonephritic kidney. As a corollary to this, it would appear that nephron groups which sustained significant structural damage disappeared from the population of functioning units.

If the experimental pyelonephritis in the present animals is analogous to human pyelonephritis, it seems reasonable that the majority of residual nephrons of the pyelonephritic kidney in man also retain their basic integrity. Moreover, this conclusion should obtain whether there be one diseased kidney or two For this reason, an attempt may be made to interpret the abnormalities in renal function and body fluid composition in bilateral renal disease within the framework of an intact nephron hypothesis.[6] The major factor in the evolution of abnormalities would consist of a progressive decrease in the population of functioning nephrons. As a consequence, all constituents of body fluids acquired at a relatively constant rate and excreted by glomerular filtration (urea, creatinine, and others) would accumulate in body fluids However, substances excreted by active tubular transport could be maintained in essentially normal concentrations if appropriate adaptive changes in renal function ensued. The fact that sodium balance, plasma sodium concentration, water balance, total body water content, potassium balance, plasma potassium concentration, and many other parameters of body fluids are often maintained within normal limits throughout the major portion of the natural history of chronic pyelonephritis is in support of this hypothesis. The major adaptation relevant to salt and water excretion consists of the ability of the residual functioning nephrons to excrete an ever-increasing fraction of the filtered sodium, chloride, and water. One functional change which could facilitate this is an increased glomerular filtration rate per nephron.[14] The present experiments suggest that this adaptation may even occur to

* In addition to the data discussed above, similar results have been obtained in experiments on chloride, potassium, and phosphate excretions by the diseased kidneys[7]

a limited degree in the pyelonephritic kidney when the contralateral kidney is intact and the internal environment normal. Presumably it could occur to a much greater degree in bilateral renal disease. The excretion of a large fraction of the filtered salt and water, moreover, would help to explain the emergence of isosthenuria in bilateral renal disease. Thus the concentrating and diluting mechanisms may continue to operate, but the great volumes of urine which pass the active sites would preclude the elaboration of maximally concentrated or maximally dilute urines.* The same adaptive changes may contribute to the inability of the patient with bilateral disease to reabsorb all the filtered sodium. Adaptive changes in nephron function may occur in response to hormonal adjustments. Among these is the decreased reabsorption of filtered phosphate which appears to be mediated in part by an increased secretion of parathyroid hormone.[10] Finally, it seems reasonable to assume that a number of other integrated adaptations occur in progressive bilateral pyelonephritis that facilitate the appropriate changes in excretion of many other solutes by the surviving nephrons.

Summary

Studies have been performed in an attempt to evaluate the functional integrity of the persisting nephrons of the pyelonephritic kidney. The experimental model has consisted of the dog with unilateral pyelonephritis and a contralateral normal kidney in which the simultaneous functions of both organs could be studied serially. The data have revealed a remarkable degree of functional ability in the residual nephrons of the pyelonephritic kidney, and no evidence has been obtained of functional abnormalities which might relate to randomly distributed structural abnormalities in the nephron architecture. On the basis of these observations, it has been suggested that the majority of nephrons in the pyelonephritic kidney are basically intact and that units which have been subjected to marked structural impairment have ceased to contribute in a detectable manner to total renal function. Finally, an attempt has been made to explain certain of the limitations in function which occur in bilateral pyelonephritis on the basis of integrated functional adaptations in nephrons, the majority of which have retained their essential functional integrity.

* The possibility exists that spatial alterations in the inner medulla of the severely diseased kidney modify the efficiency of the concentrating mechanism, but this does not appear to be the dominant factor.

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*Prolonged Observations on a Group of Patients with Acute Urinary Tract Infections**

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It is assumed by most authors that chronic pyelonephritis is the result of long-standing or repeated acute episodes of bacterial infection. It is important to remember, however, that most clinico-pathologic correlations of pyelonephritis were carried out at a time when the bacteriologic examination of the urine was qualitative and therefore difficult, if not impossible, to interpret.[3, 4] Furthermore, no consideration was given to prior instrumentation of the urinary tract, a procedure now known to be capable of introducing into urine bacteria which may persist for prolonged periods of time.

The present report is the beginning of an effort to carry out a long-term study of a group of adult patients with urinary tract infections employing careful quantitative bacteriologic methods. In this way it is hoped that the physiologic and morphologic consequences of these infections can be defined, and in addition therapeutic principles firmly established. This study is now in its fourth year. The materal being presented today deals with data collected during the first two and one-half years, from July of 1956 to December of 1958.

The 142 patients chosen for discussion include all those encountered with bacterial (excluding tubercle bacillus and gonococcus) infections of the urinary tract, without evidence of disease of the anterior urethra or prostate, in whom no obstruction to the flow of urine beyond the renal papilla was know to exist. At the time of first observation, all but 12 had symptoms of urinary tract infection. These 12 were referred to the clinic because a positive urine culture had been noted in the course of other examinations. Several of the patients in the latter group have since had symptomatic infections. Such cases are included in this study since it became clear that a positive urine culture in the absence of symptoms represents a stage of urinary tract infection which might either follow or precede acute symptomatic infections. Additional evidence

* Supported by U.S. Public Health Service grant E-967(C).

that these patients do not differ significantly from the symptomatic group is found in the similarity of their response to treatment.

Patients considered to have urinary tract infections demonstrated at some point in their disease at least 10^5 bacteria per milliliter in a clean *voided* specimen of urine in two or more consecutive cultures.[1] A urine culture was considered negative when it contained 10^3 or fewer bacteria per milliliter. A single positive culture was accepted as evidence of infection only when accompanied by typical clinical acute illness. Efforts to differentiate cystitis, ureteritis, pyelitis, and pyelonephritis from each other resulted in such difficulties that these terms were abandoned in favor of the generic term, urinary tract infection. The term chronic pyelonephritis from this point on will be avoided rather than defined.

The age and sex distributions of the total group of 142 patients are illustrated in Figure 1: 91 per cent of the patients are female; 72.5 per cent of the group are between the ages of 20 and 49 years. The great preponderance of cases in the young female supports the view that sexual activity and childbearing may be important contributing factors in this disease.

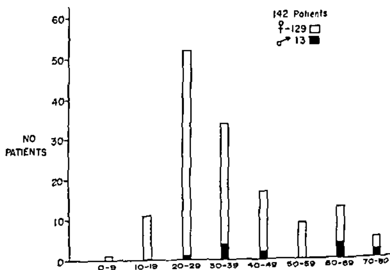


FIGURE 1 Age and sex distribution of total patient group.

Thirty-one per cent of the entire patient group had a blood pressure which exceeded 140/90 mm. Hg (Figure 2). This degree of hypertension was found in 17.3 per cent of patients under 40 and in 61 per cent of

patients over this age If one confines the definition of hypertension to those patients whose diastolic blood pressure equaled or exceeded 100 mm. Hg, the figures become 15 3 per cent below 40 and 43 2 per cent over 40. This incidence of high blood pressure is considerably higher than would be expected in the normal population,[2] particularly in patients less than 40 years of age. Of further interest is the fact that 10 of the 19 hypertensive

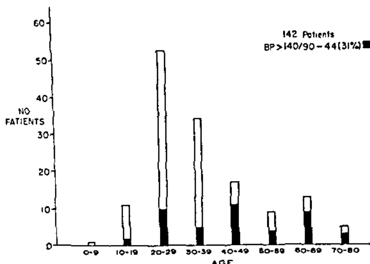


Figure 2 Distribution of high blood pressure in total patient group

patients over age 40 and 10 of the 15 under 40 gave no history of prior urinary tract infection

In addition, persistent abnormalities of renal function as measured by the blood nonprotein nitrogen and the 2-hour excretion of phenolsulfonphthalein were detected in 15 patients, most of whom were regarded as having their first urinary tract infection.

These findings indicate that hypertension and renal functional impairment are common in patients with infection of the urinary tract, even in those thought to be infected for the first time. It is possible that these patients had been harboring unrecognized infections for many years. The present data, however, serve to emphasize the difficulties encountered in accepting this statement, since an alternative possibility is that susceptibility to infection may be increased in patients with hypertension or renal scarring.

Pyelography was performed in 73 patients, most of whom had not responded to antibiotic therapy. Fifteen had changes ascribed to pyelo-

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Some Clinical Aspects of Chronic Pyelonephritis

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Most of the important clinical features of chronic pyelonephritis have been well described in the literature of recent years, beginning two decades ago with the pioneering studies of Longcope[4] and of Weiss and Parker.[6] Many in this audience have made significant contributions to our present understanding of this disease. It seems quite unnecessary, therefore, to review again those aspects of the subject which must by now be common knowledge I shall instead confine myself to a few observations on certain diagnostic aspects of the disease which may not be widely appreciated, but which have considerable practical significance.

The data to be presented are based on 29 patients with morphologically demonstrated chronic pyelonephritis personally observed during the past 5 years in the medical wards and clinics of the Massachusetts Memorial Hospitals These cases were selected from among a much larger clinical experience, solely on the basis of the fact that the diagnosis in each case had been established by open surgical biopsy, nephrectomy, or autopsy. In the great majority of cases the histologic diagnosis was active chronic pyelonephritis, or acute and chronic pyelonephritis, often together with varying degrees of nephrosclerosis Cases with other complicating renal diseases were excluded

Table I summarizes some of the general clinical features of this group of 29 patients when first seen In 22 the disease was "primary," that is to say, uncomplicated by obvious obstructions, anomalies, calculi, or the like This group was in general young and overwhelmingly female. Most were hypertensive, blood nitrogen levels were elevated in over half, and 5 were in the terminal stages of uremia Of the 7 patients whose disease was apparently secondary to obstruction or stone, 5 were male, 4 had hypertension, and 3 were uremic

The first point I should like to make concerns the x-ray appearance of the kidneys in the nonobstructive group Satisfactory intravenous or retrograde pyelograms were obtained in 17 of these patients and in all but 2 some abnormality in the collecting system of one or both kidneys was evident In some cases the changes were quite minimal and might easily escape notice They were confined to the calyceal system and

The final point I wish to make concerns the significance of the presence of cellular casts in the spun sediment. It is commonly stated that the urine sediment in chronic pyelonephritis is notably free of casts, at least until renal damage is far advanced. However, careful examination of the spun sediment in the fresh unstained condition revealed cellular casts in

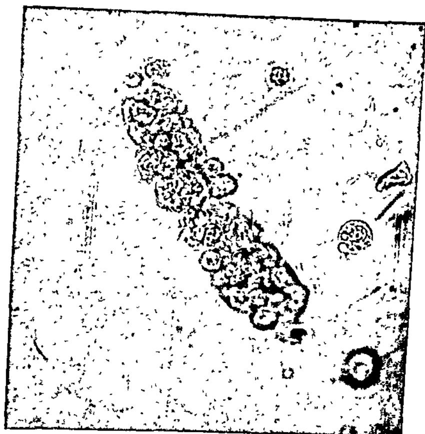


FIGURE 1 A well-preserved leukocyte cast.

the initial one or two urines in 25 of the 29 patients in this group. In 4 of the 5 patients with initially negative urines, subsequent examination ultimately revealed these casts.

One such cast is illustrated in Figure 1. In some instances, such as this, the cells were well-preserved and were unmistakably leukocytes In other cases, as in Figure 2, a varying degree of degeneration had occurred and the identification of the cellular elements was somewhat uncertain. Sometimes the casts were of unexpected shape and size, due to folding

or fragmentation. Occasionally elements were encountered which could have been either fragments of cellular casts or densely packed clumps of degenerating cells For the purposes of this study, these elements were not adjudged actually to be casts. However, experience has shown that the isolated finding of such cellular fragments in urines otherwise free

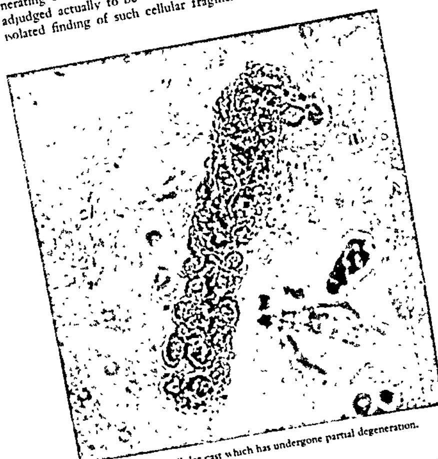


FIGURE 1 A cellular cast which has undergone partial degeneration.

of cells and clumps of cells has almost the diagnostic significance of the well-preserved cellular casts

It must be emphasized that except in an occasional patient with gross pyuria and clinically active infection, relatively few cellular casts are to be found in any given sediment Often we have noted only one or two on an entire slide In the absence of severe pyuria or advanced renal damage the sediment in chronic pyelonephritis is quite sparse, often con-

sisting solely of a few cells, some unidentifiable cellular debris, and a rare cellular cast. Unless pains are taken to concentrate all the available sediment into the drop of urine examined, and unless a fresh specimen is examined with the most meticulous care, the cellular casts will be missed When gross pyuria or renal failure supervenes, cellular casts may be obscured by the large number of cells, clumps, and other types of casts Also, in some grossly infected very alkaline urines all casts may dissolve very rapidly. These considerations may perhaps explain why the diagnostic significance of the cellular cast in this disease has not been more widely appreciated.

Time does not permit further expansion of this subject, but I do not wish to imply that I believe leukocyte casts to be specific for pyelonephritis. They most certainly are not. Nevertheless, these data do suggest that proper examination of the unstained urine sediment will aid considerably in the recognition of this disease. As our clinical experience has widened we have come to rely on this to an increasing extent.

SUMMARY

A study of 29 cases of morphologically established chronic pyelonephritis revealed that significant abnormalities in calyceal outline could be detected by pyelography in almost all. Initial bacterial counts were within normal limits in more than half, but leukocyte casts were found on first examination in the urine sediment of 25 cases. More attention should be paid to the diagnostic value of the x-ray and the sediment examination in this disease.

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GENERAL DISCUSSION

DR. JACKSON: I should like to commend Dr. Brod and Dr. Relman on their very clear exposition of the clinical diagnosis of chronic pyelonephritis. I do not share entirely Dr. Freedman's diffidence in arriving at a clinical diagnosis of this entity. I think we should recognize it, and I think it can be diagnosed clinically with accuracy.

Dr Brod has gone over the diagnostic data very well, and our findings and experience agree with his. Proteinuria certainly is slight in chronic pyelonephritis and is nearly always below 3 Gm. per day. Red cells almost invariably indicate stones, congenital anomalies, or other underlying disease. Pale-cell pyuria is usually present. Casts are infrequent. Our clearance studies in patients with pyelonephritis fit well with Dr. Bricker's model

Another critical point Dr Brod made is that one must distinguish obstructive and nonobstructive pyelonephritis and acute and chronic pyelonephritis I am talking about clinical recognition of nonobstructive chronic pyelonephritis We have recently reviewed 119 autopsies of patients with this disease, and they show precisely the conditions Drs. Brod and Relman have pointed out

Upon the basis of this group and the results from our autopsy series at large, people with nephrosclerosis did not have an increased susceptibility to pyelonephritis. About 10 per cent of persons with arteriolonephrosclerosis as a pathologic diagnosis had pyelonephritis, and this was the incidence of pyelonephritis for the entire group. The same is true for the patients with glomerulonephritis

The occurrence of hypertension with pyelonephritis was again clearly evident The hypertension was usually mild, with grade I or II retinopathy. Uremia was an outstanding part of the clinical picture, and as Dr. Brod pointed out, the patients usually died free of edema

Chronic nonobstructive pyelonephritis is a clear-cut clinical syndrome that accounts for the majority of deaths of people in uremia, and usually it can be identified clinically

DR. M[illegible]: I should like to return to some of the earlier papers and discuss a point relating to disturbances of function There is one point which is implicit in several of the papers, and this perhaps warrants emphasis It applies to the normal kidney and perhaps even more to the pyelonephritic kidney.

We have heard mention of the disproportionate loss in concentrating power of the pyelonephritic kidney. As a result of the scheme which Dr. Wirz has outlined, a major factor in concentrating power can be localized to the medulla, and this presumably is the initial seat of the pyelo-

free water clearance or the $Tm^{c}_{H_2O}$), *the maximum concentration of* the urine for any given rate of solute excretion, corrected to the normal mass, is always much less in chronic renal disease, and particularly in chronic pyelonephritis, than it is in the normal.

We and Dr. Merrill in Boston have been unable, by lowering the rate of solute excretion in the chronically diseased kidney, to improve the concentrating capacity. We have been invariably able to improve the renal diluting capacity by lowering the rate of solute excretion. I think this is further evidence that there is a true defect in concentration, but possibly not a true defect in diluting capacity.

DR. BROD I have given great thought to the differences in our results from those of Dr Bricker. I think the differences are only seemingly great.

Dr. Bricker has devoted most of his work to proximal functions. These functions are not very much disturbed in chronic pyelonephritis. There is some disturbance of these functions in humans. It seems to follow from the work of Alexandrov in Warsaw, relating the Tm_{PAH} to the rate of glomerular filtration.

As for the proportion between the degree of constriction of concentrating power and of glomerular filtration, it was pointed out by Dr. Kleeman and by Dr. Prát, my associate, that with a similar proportionate restriction of both the functions, there is a greater restriction of concentrating power than of glomerular filtration rate.

Concerning Dr. Bricker's point that the restriction of concentrating power might be simply another adaptive change, and that the glomerular filtration rate was not changed owing to an increase in size of the nephrons, I think there is one thing that speaks against this being so. In subjects with acute pyelitis or in subjects with an obstruction of a ureter lasting for several hours, the glomerular filtration rate immediately after removal of the obstruction will be normal, or at least it will be the same as it will be found five or six weeks later, and the concentrating power will more slowly return to normal.

There is one question I should like to direct to Dr. Wirz. When fifty or sixty years ago change in the concentrating ability of the kidneys was discovered, it was assumed that dilution and concentration were two facets of the same function. This assumption has been repeated again and again in the clinical literature until 1950.

My question is this: Are all the restrictions of the concentrating power due to the same defect? In pyelonephritis the defect is in concentrating power, showing there must be a reduction of sodium, obviously, somewhere in the ascending loop of Henle. This functions well. What happens afterward is that somewhere in the distal tubule or still further in the

collecting duct there is no response to ADH and no extraction of water in excess of sodium.

In cases of glomerulonephritis, as mentioned by Dr. Kleeman, we have an isosthenuria. If Dr Wirz's findings can be transferred to an abnormal kidney, then one must have extraction of sodium in the loop of Henle, but what happens is a failure of extraction of water in excess of sodium in the collecting duct.

So I think we probably shall have to study in more detail the different patients with different changes of concentrating power to see whether we will be able to locate the defect in more detail.

Dr Keitel Dr Brod stated that many patients had obstructive lesions demonstrated as part of the clinical problem. Dr. Relman's patients, I believe, were picked so that no patients with known obstruction were studied, yet the patients showed very similar findings. Therefore, in view of Dr Murphy's findings, I should like to ask if any of the subtle techniques which might pick up obstruction were used in Dr. Brod's patients who did not show obstructive phenomena by gross methods. The same might be asked of Dr. Relman's cases, particularly whether there was any evidence of reflux.

Dr Relman Six of our patients, we think, did have obstruction, and we didn't use any "subtle" methods for diagnosing the presence or absence of obstruction We used conventional urological and x-ray techniques Our impression is that the only difference between those with and without obstruction is that the people who have obstruction are more apt to have pyuria and bacteriuria

Dr Sickles Dr. Kass, you stated that urine can be stored for 48 hours in the refrigerator without significant change in the bacterial count. I wonder if this is true with reference to the sediment changes, if not, is there any method than can give us the advantages of the analysis of a fresh specimen and still allow us a little more time for examination?

Dr Kass The answer is no I will go into it this afternoon, so perhaps we can wait until then

Dr Pirani I should like to comment briefly on the very high correlation between clinical diagnosis and pathologic findings in the data of Dr Brod, which contrasts with the much less impressive correlations between clinical and laboratory data and the autopsy, surgical biopsy, and percutaneous biopsy findings in the data of others.

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DR. PIRANI: I should like to comment briefly on the very high correlation between clinical diagnosis and pathologic findings in the data of Dr. Brod, which contrasts with the much less impressive correlations between clinical and laboratory data and the autopsy, surgical biopsy, and percutaneous biopsy findings in the data of others.

If I recall correctly, Dr. Brod showed 94 per cent correlation between clinical and pathologic findings. This might be explained by the fact that he has applied excellent and rigid clinical criteria to the diagnosis of pyelonephritis. Also, 75 per cent of his cases had some evidence of obstruction and 25 per cent apparently did not. I wonder if there was a less good correlation in those cases which had no obstruction.

Actually, the problem is not so much with the patients who have evidence of obstruction as with those patients in whom there is no such evidence, and in whom (as often happens) at autopsy or renal biopsy we find chronic pyelonephritis by classic histologic criteria. These patients often do not have a definite picture of pyelonephritis clinically.

I should like to make a brief remark about colloid casts. Obviously there has to be a discrepancy between the number of casts in the kidney itself and in the urinary sediment in pyelonephritis. Most pathologists will interpret these large casts in the dilated tubules as a result of inspissation of protein associated with intrarenal obstruction. I don't believe these casts can float down through the tubular tract.

Finally, I should like to say a word in favor of the nephron as a functional unit. Various segments of the nephron have different functions, but if there is damage to one of these segments, it is probable that the others will not function very well either. This would, of course, be extremely difficult to prove.

DR. MICHIE: Previously we showed in patients with asymptomatic chronic unilateral pyelonephritis that:

(1) The renal clearance equilibrium time was essentially the same for both the nondiseased and the diseased kidney. Thus the inflammatory reaction in the diseased kidney does not delay the excretion of urine.

(2) When the maximal tubular secretion of p-aminohippurate (Tm_{PAH}) for a single kidney was greater than 10 mg. per minute, then:

(*a*) The function of the individual nephrons in the nondiseased and diseased kidney was statistically identical. Thus an equivalent degree of [illegible] all functioning nephrons, including those [illegible] The decrease in total function of the [illegible] related to the total number of nephrons

(*b*) Glomerular filtration rate (GFR) per unit tubular function (GFR/Tm_{PAH}) for both the nondiseased and diseased kidney was essentially the same as that for the normal subject.

(*c*) Effective renal plasma flow (ERPF) per unit tubular function ($ERPF/Tm_{PAH}$) for both the nondiseased and diseased kidney was significantly less (79.6 per cent) than the value for the normal subject. Thus,

while they possess no glomerular proximal tubular imbalance, the pyelonephritic kidney and its nondiseased mate are definitely ischemic when compared with the renal plasma flow of normal subjects.

(*d*) The concentration of the test substances – inulin, p-aminohippurate, phosphate, chloride, and pH – in the simultaneously formed urine by the nondiseased and the diseased kidney was identical: within ±5 per cent of their mean value in 70 per cent of 140 determinations. Thus, if the maximal tubular excretion of p-aminohippurate is greater than 10 mg. per minute, we cannot find any impairment in concentrating ability of the pyelonephritic kidney when it is compared with its nondiseased mate.

Our data and conclusions are supported by Dr. Bricker's but are at variance with Dr. Brod's.

SUMMATION

Louis G. Welt
(Chapel Hill, North Carolina)

Dr Giebisch and his colleagues have contributed some direct observations with respect to the problem of localization, or the topophysiology, of urinary acidification Once again, some hitherto held ideas have been literally punctured. These direct observations and those to which Dr. Giebisch alluded, made by Dr. Gottschalk and Dr. Ullrich, have provided some important landmarks in our information concerning this important problem of renal physiology.

Dr. Wirz has described for us one of the revolutions in renal physiology, beginning with the suggestion of Hargitay and Kuhn, his own brilliant work, and that of Dr. Gottschalk. It would be unwise to state that we know in detail just how the urine is concentrated, but we certainly have a broad outline, which is now bounded by some clear and unassailable data. It is, perhaps, trite, since this thought was forcibly brought to our attention yesterday by Dr. Oliver and others, but the story of these developments in terms of the concentrating mechanism has made it abundantly clear that the functionalist and the structuralist had better become more closely and more continuously acquainted. The failure to recognize that the peculiar morphology of the nephron might have some significance with respect to its function presumably delayed the recognition of the nature of the concentrating mechanism.

Dr. Epstein and Dr. Carone have described lesions in the collecting ducts that are correlated with a concentrating defect. The disturbance responsible for the concentrating defect has not yet been adequately pinpointed, and I do not think that the observation of a decreased concentration of sodium in medullary water can help us to discriminate between

V

The Microflora in Pyelonephritis

Chairman. GEORGE GEE JACKSON, M.D. (*Chicago, Illinois*)

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The Taxonomy of Enterobacteriaceae

PHILIP R. EDWARDS, Ph.D., and WILLIAM H. EWING, Ph.D.
(Atlanta, Georgia)

In the formal classification of bacteria one encounters two separate problems, nomenclature and taxonomy, and all too often these are confused. As regards the Enterobacteriaceae, this confusion of nomenclature and taxonomy has added to our difficulties in translation of a logical taxonomic scheme into a formal classification by the nomenclaturist. Cowan[3,4,5] published adequate biochemical descriptions of the various groups of enteric bacteria and attempted to reconcile the differences of the taxonomist and nomenclaturist through a dual system of nomenclature, one for the specialist who works intensively with the bacteria, the other for the purist who would set up a system based upon the rules of nomenclature. Here we are concerned only with the taxonomy of the bacteria and their separation into readily identifiable groups.

Classifications of enteric bacteria which recently have attracted the greatest attention are those of Breed, Murray, and Smith[1] in *Bergey's Manual* and of Kauffmann.[16] The tribes or main divisions and the genera or groups recognized in these two classifications are given in Tables I and II respectively. While we do not wish to discuss these classifications in detail, the following remarks seem justified: In dividing the family primarily on lactose fermentation and secondarily on pigment production, Breed, Murray, and Smith have fallen into two oft-committed errors. First, every group which characteristically ferments lactose contains components which attack the sugar rapidly, others which ferment it slowly, and still others which fail to ferment it. Second, the majority of the members of the one group which is characterized by pigment production fail to produce pigment when tested by the usual methods. In dividing the organisms on the basis of lactose fermentation these authors have placed together groups having diverse characteristics and separated groups which have close biochemical and serologic relationships. The establishment of a single genus composed of diverse forms simply upon the basis of delayed fermentation of lactose is completely unjustified.

The classification of Kauffmann (Table II), while differing somewhat in nomenclature, from a taxonomic standpoint more closely resembles that advanced by Ewing and Edwards,[7] although primary division of the

TABLE I. Classification of Enterobacteriaceae
Bergey's Manual, 7th Edition

Tribes	Genera
Escherichia	Escherichia Aerobacter Klebsiella Paracolobactrum Alginobacter
Erwinieae	Erwinia
Serratieae	Serratia
Proteae	Proteus
Salmonelleae	Salmonella Shigella

bacteria is based upon different criteria. Considering the technical difficulties presently involved in applying the glutamic acid decarboxylase test in the average laboratory, it seems best not to use this test as a primary method of dividing the groups Further, the Citrobacter group seems more closely allied biochemically to the Salmonella-Arizona complex than to the groups among which it is included in the Kauffmann classification. However, it should be emphasized that the classifications

TABLE II. Classification of Kauffmann, 1956

Groups of Enterobacteriaceae	KCN	Glutamic Acid Decarboxylase
I Salmonella Arizona	−	−
II Shigella Escherichia	−	+
III Citrobacter Klebsiella Cloaca Hafnia Erwinia Serratia	+	−
IV Proteus Morganella Rettgerella Providencia	+	+

proposed by Cowan, by Kauffmann, and by us are basically similar, and that the taxonomic groups recognized are identical although the methods of arriving at group differentiation and the nomenclature involved differ in each instance.

A classification which seems logical to us, and which is based upon the principles set forth by Kauffmann, Edwards, and Ewing,[11] is presented in Tables III to XI inclusive. The principal divisions and groups recognized are given in Table III and the criteria for recognition of the principal

TABLE III The Principal Divisions and Groups of Enterobacteriaceae

Principal Divisions	Groups
Shigella-Escherichia	Shigella Escherichia (*E coli*, including Alkalescens-Dispar)
Salmonella-Arizona-Citrobacter*	Salmonella Arizona Citrobacter* (including Bethesda-Ballerup)
Klebsiella-Aerobacter-Serratia	Klebsiella Aerobacter Hafnia Serratia
Proteus-Providence	Proteus Providence

* Formerly *Escherichia freundii*

divisions in Table IV It will be noted that the principal divisions are based upon the IMViC reactions, hydrogen sulfide production, urease production, ability to grow in Moeller's KCN medium, and production of phenylalanine deaminase. These tests were selected because they can be used to divide the family into broad categories, the individual groups of each of which possess many common biochemical properties and, in many instances, close serologic relationships. Conversely, the inclusion of quite diverse forms in the same principal division is avoided.

The Shigella-Escherichia division is composed of organisms all of which are methyl red positive and Voges-Proskauer, citrate, hydrogen sulfide, urease, KCN, and phenylalanine negative. The Shigella and Escherichia groups, as a rule, are separated without difficulty by the tests outlined in Table V Certain *Escherichia coli* cultures are nonmotile, or fail to ferment lactose promptly, or are anaerogenic, but it is extremely unusual to find Escherichia cultures which conform in all respects to the properties

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TABLE IV. Differentiation of the Principal Divisions Within the Enterobacteriaceae by Biochemical Methods

Reactions of Typical Cultures

Substrate or Test	Shigella-Escherichia Division	Salmonella-Arizona-Citrobacter Division	Klebsiella-Aerobacter-Serratia Division	Proteus-Providence Division
Indol	d	−	−	d
Methyl red	+	+	−	+
Voges-Proskauer	−	−	+	−
Simmons' citrate	−	+	+	d
Hydrogen sulfide (TSI)	−	+	− or (+)	d
Urease	−	−	− or (+)	d
KCN	−	d	+	+
Phenylalanine	−	−	−	+

Note: *S typhi*, *S paratyphi A*, and some of the rare types are citrate negative *S paratyphi A* and other rare types are hydrogen sulfide negative

+ positive in one or two days
− negative
(+) delayed positive
d different biochemical types

TABLE V The Shigella-Escherichia Division

Substrate or Test	Shigella Group	Escherichia Group
Gas from glucose	−*	+ (−)
Lactose	−*	+ or x
Salicin	−	d (approx 80% +)
Motility	−	+ (−)
Lysine decarboxylase	−	+
Mucate	−	+ (−)
Christensen's citrate	−	+ (−)

[illegible] and

− negative
+ (−) majority of strains positive, [illegible] es occur
x late and irregularly positive
d different biochemical types

* Certain b[illegible] 6 form [illegible] tures ferment lactose slowly

attributed to the Shigella group in Table V. Certain ancillary tests such as fermentation of dulcitol, maltose, xylose, rhamnose, and sorbitol also are helpful in identification of doubtful cultures.

The Salmonella-Arizona-Citrobacter division is composed of organisms which are indol, Voges-Proskauer, urease, and phenylalanine negative and methyl red, citrate, and hydrogen sulfide positive. The Citrobacter group is distinguished by its failure to produce lysine decarboxylase and its ability to grow in KCN medium (Table VI). The Salmonella and Arizona

TABLE VI THE SALMONELLA-ARIZONA-CITROBACTER DIVISION

Substrate or Test	Salmonella Group	Arizona Group	Citrobacter Group
Lactose	−	+ or x	+ or x
Dulcitol	+	−	d
Gelatin	−	(+)	−
KCN	−	−	+
Lysine decarboxylase	+	+	−
Malonate	−	+	−

Note. The majority of salmonellae ferment dulcitol promptly, but *S typhi*, *S pullorum*, *S paratyphi A*, *S cholerae suis*, and a few others do not do so Members of the Arizona group are uniformly negative on this substrate *S paratyphi A* is lysine negative

+ positive in one or two days
− negative
(+) delayed positive
x late and irregularly positive
d different biochemical types

groups may be separated through their action on dulcitol, gelatin, and malonate The rapid gelatin liquefaction test of Kohn as modified by Lautrop [13] increases the value of gelatin as a differential medium in this instance. Tests for the rapid utilization of D-tartrate, citrate, and mucate by the method of Kauffmann and Petersen [12] also are of value in distinguishing Salmonella, Arizona, and Citrobacter cultures as shown in Table VII Many Citrobacter strains ferment dulcitol promptly and this aids in distinguishing them from Arizona cultures, which are uniformly dulcitol negative. When the biochemical properties of well-known Salmonella serotypes are considered, little difficulty is encountered in the separation of the three groups which compose this division.

The Klebsiella-Aerobacter-Serratia division is made up of forms which are indol, methyl red, hydrogen sulfide, urease, and phenylalanine negative and Voges-Proskauer, citrate, and KCN positive. The delayed positive urease reactions which occur in this division are not to be confused with the strong, rapid production of urease by Proteus cultures. It will be

mann While some confusion of nomenclature exists in this area, from a taxonomic standpoint the organisms easily can be distinguished from each other and from the Klebsiella group. Subgroup C is synonomous with *Aerobacter liquefaciens* of Grimes and Hennerty[8] and apparently is a truly psychrophilic group of bacteria. Their whole biochemical activity is greater at 22° C. than at 37° C. Subgroup C liquefies gelatin rapidly and fails to ferment rhamnose, whereas rhamnose is fermented promptly by subgroups A and B, and gelatin, when liquefied, is attacked slowly. Gas production from cellobiose is delayed in subgroup C but prompt in subgroups A and B As shown in Table X, subgroup C is differentiated

TABLE X. Differentiation of Aerobacter Subgroup C and the Hafnia and Serratia Groups

Substrate or Test	Aerobacter Group Subgroup C		Hafnia Group		Serratia Group
	37° C	22° C	37° C.	22° C.	
Gas from glucose	+	+	+	+	d
Gas from glycerol	d	(+)	+	+	–
Gas from inositol	+	+	–	–	–
Gas from cellobiose	d	(+)	+	+	–
Sorbitol	+	+	–	–	+
Arabinose	+	+	+	+	–
Raffinose	+	+	–	–	–
Rhamnose	–	–	+	+	–
Gelatin		+		–	+

Note When gas is formed from glucose by Serratia, the volumes are small (10 per cent or less)

+ positive in one or two days
– negative
(+) delayed positive
d different biochemical types

rom Hafnia by delayed gas production from glycerol and cellobiose, ermentation of sorbitol, raffinose, and rhamnose, and liquefaction of elatin It is distinguished from Serratia by volumes of gas produced rom glucose, production of gas from glycerol, cellobiose, and inositol, nd fermentation of arabinose and rhamnose. The Hafnia and Serratia roups are differentiated by volumes of gas produced from glucose, roduction of gas from glycerol and cellobiose, fermentation of sorbitol, rabinose, and rhamnose, and by liquefaction of gelatin. It should be mphasized that Serratia cultures often are anaerogenic, and when gas s produced from fermentable substances the volumes are small, not xceeding 10 per cent.

The Proteus-Providence division is composed of organisms which are methyl red, KCN, and phenylalanine positive. The Voges-Proskauer test, with rare exceptions in *Proteus mirabilis*, is negative This division has been treated differently by various workers. Cowan and Breed, Murray, and Smith place all the forms in the Proteus group, making the Providence group a species of Proteus, *Proteus inconstans*. Kauffmann divides the Proteus group into Proteus (containing *Proteus mirabilis* and *Proteus vulgaris*), Morganella, and Rettgerella. He also retains the Providence group Ewing suggested that the genus *Proteus* might be confined to *Proteus mirabilis* and *Proteus vulgaris* while *Proteus rettgeri*, *Proteus morganii*, and the Providence group might be assigned to the genus *Morganella* In the present proposal the traditional form of the Proteus group as defined by Rustigian and Stuart [16] is retained and the Providence group included as a part of the division The two groups are separated upon the basis of urease production Further, additional clear-cut differences exist between the vulgaris, mirabilis, and morganii subgroups and the Providence group. It would appear from Table XI that urease

TABLE XI THE PROTEUS-PROVIDENCE DIVISION

Substrate or Test	Proteus Group				Providence Group
	vulgaris	mirabilis	morganii	rettgeri	
Mannitol	−	−	−	+	d
Indol	+	−	+	+	+
Simmons' citrate	d	d	−	+	+
Hydrogen sulfide (TSI)	+	+	−	−	−
Gelatin	+	+	−	−	−
Urease	+	+	+	+	−

\+ positive in one or two days
− negative
d different biochemical types

production was the only reliable method of differentiating the rettgeri subgroup from the Providence group, but actually other differences exist Providence cultures which ferment mannitol promptly are rare, whereas mannitol is fermented regularly by rettgeri cultures Further, whereas the rettgeri strains ferment both adonitol and inositol, it is unusual to find Providence cultures which attack both substances.

The foregoing discussion is based upon the biochemical behavior of typical cultures of the various known groups. It is obvious that atypical cultures occur which may not be classifiable readily in any of the groups delineated. There is a strong probability that additional groups exist and

DESIGNATED DISCUSSION

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Many of the groups and species of bacteria so competently discussed by Edwards and Ewing are responsible for infections of the urinary tract. Most commonly encountered is *Escherichia coli*. *Aerobacter aerogenes* and related organisms as well as members of the genera *Proteus* and *Pseudomonas* are isolated less frequently. A similar, although not identical, pattern of distribution of various bacteria in infections of the urinary tract (see Tables I and II) was observed in our laboratories in two rather

TABLE I. MICROORGANISMS ISOLATED FROM URINE OF 100 CHILDREN WITH INFECTIONS OF THE URINARY TRACT

Microorganisms	Acute Cases	Chronic Cases
Escherichia coli	28 (34 1%)	11 (36 6%)
Aerobacter species	10 (12 2%)	4 (13 3%)
Proteus species	9 (10 9%)	5 (16 6%)
Slow or non-lactose-fermenting bacilli	6 (7 3%)	1 (3 3%)
Shigella alkalescens	1 (1 2%)	0 (0%)
Pseudomonas aeruginosa	3 (3 6%)	2 (6 6%)
Staphylococcus	16 (19 5%)	4 (13 3%)
Enterococcus	2 (2 4%)	1 (3 3%)
No organisms cultured	7 (8 5%)	2 (6 6%)
Total	82	30

TABLE II. MICROORGANISMS ISOLATED FROM URINE OF 100 ADULTS WITH MALIGNANCIES AND INFECTIONS OF THE URINARY TRACT

Microorganisms	Acute Cases	Chronic Cases
Escherichia coli	2 (28 5%)	34 (28 3%)
Aerobacter species	0 (0%)	18 (15 0%)
Proteus species	0 (0%)	13 (10 8%)
Slow or non-lactose-fermenting bacilli	1 (14 2%)	5 (4 2%)
Pseudomonas aeruginosa	0 (0%)	18 (15 0%)
Staphylococcus	2 (28 5%)	13 (10 8%)
Enterococcus	1 (14 2%)	7 (5 8%)
Nonhemolytic streptococcus	0 (0%)	3 (2 5%)
Pneumococcus	0 (0%)	1 (0 8%)
No organism cultured	1 (14 2%)	8 (6 6%)
Total	7	120

diverse groups, namely, children and adults with malignancies (Work done in collaboration with Dr E. Rajnovich.) But the characterization of these microorganisms should go still further.

On the basis of their antigenic composition the species of gram-negative bacilli are subdivided into numerous serogroups and serotypes The question presents itself as to whether differences exist in the pathogenic potential of various species and various serotypes This question is raised because it has been clearly established during the past decade that a few special serotypes of *E coli*, in contrast to numerous others, are endowed with the hitherto unexplained capacity of producing enteritis in young infants.[5] Furthermore, Kauffmann[6] has shown that certain members of the *E coli* group are encountered more frequently in appendicitis than in the feces of healthy individuals, and he has advanced the theory of appendicitis as a true infectious disease. Obviously, in the interpretation of the distribution of various gram-negative bacilli in infections of the urinary tract, one has to consider the frequency of occurrence of these microorganisms in the sources of infection. Several studies in this direction were made prior to the elucidation of the antigenic structure of *E. coli* Since that time, Vahlne[9] has studied this problem and observed that no particular serologic type of *E. coli* occurs exclusively in infections of the urinary tract Nevertheless, the urinary material showed a certain preponderance of group 4 strains He has also confirmed the previous observation that hemolytic strains are encountered more frequently in lesions than in the feces of healthy individuals The main question to be answered in the future is whether certain types have a greater nephropathogenic potentiality than others, or, put in another way, whether the distribution of different organisms in whatever serves as source of infection is different from that in the urinary tract

From a practical point of view, Dr Edwards' classification is eminently sound, and perhaps all of us should adopt it in our laboratories, so that we shall speak one and the same language. Of course, it still does not make possible the wish of some clinicians, namely, to have the results of a culture before the specimen is received

Perhaps the time is here when, for a better understanding of *ecology* and *pathogenesis*, clinical investigators and bacteriologists together should consider such growth factors, growth inhibitors, and substrates for enzyme action as occur *in vivo* to supplement the many artificial indicators now used for identification

Consideration must be given also to the number of bacteria present in freshly obtained urine. In our laboratories, at the suggestion of Dr. M. I. Rubin, we have carried out quantitative analyses routinely for more than five years. In our experience properly obtained specimens contain either only relatively few colonies per milliliter (up to a few hundred) or many

(5) Finally, one may ask the question as to the presence of antibodies in the urinary tract, for example in the plasma cells of infected kidneys, in relation to the pathogenesis and course of pyelonephritis. The role of *local* antibodies in the *intestinal tract* has been clearly established.[1] Clearly, much work remains to be done.

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*The Microflora of the Urinary Tract**

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(Houston, Texas)

The evidence obtained from carefully cultured bladder urine indicates that the urinary tract is normally sterile above the urethrovesical junction. In a study of 29 living patients without evidence of urinary tract infection, the urine obtained from percutaneous needle aspiration of the bladder was sterile in all but five.[4] Of these five patients, one had one colony per milliliter of *Bacillus subtilis*, one had eight colonies per milliliter of enterococci, and three others had two, four, and six colonies per milliliter of *Staphylococcus aureus*. At least some of these cultures might well have been contaminants coincident to the procedure. When urine was collected from these same patients by catheterization and from the middle of the voided specimen, the frequency with which bacteria could be cultured and the number of bacteria cultured increased considerably, indicating that the urethra is often not sterile in normal patients (Figure 1). The species obtained from the catheterized and midstream voided specimen were *Staphylococcus aureus*, enterococci, *Escherichia coli*, *Proteus* species, diphtheroids, and *Candida albicans*, the organisms ordinarily cultured from the skin in the perineal region.

The microflora of the diseased urinary tract is more difficult to assess because of the lack of precise methods of determining the presence or absence of infection of the kidney, ureters, or bladder. It seems well established that when the urine contains large numbers of organisms (greater than 10,000 organisms per milliliter) significant infection occurs and it should be treated.[1, 2, 3] Even under these circumstances, however, one cannot always be sure that it is the kidney and not the bladder, or even the prostate, that is infected.

In an effort to further elucidate the problem of the microflora of pyelonephritis, multiple quantitative cultures of the urine were carried out in 121 patients. Some of these patients had signs and symptoms

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us to provide the most significant information with the least effort although there were false negatives in the 300 colonies per milliliter range

Culturing the urine specimens in the thioglycollate broth almost always revealed growth when the urine was obtained by the midstream voided technique. The overgrowth of contaminants was so great that this method of culturing was considered worthless unless the specimen was obtained by bladder aspiration or careful catheterization.

Percutaneous needle biopsy of the kidney was carried out by Dr. E. M. Ory one or more times in 51 patients who had clinical evidence of pyelonephritis or who had biopsies for some other reason and were found to have histologic evidence of pyelonephritis. Both kidneys were biopsied in six patients and one patient had a congenital absence of one kidney More than one biopsy of the same kidney was performed in five patients

Since many of the patients studied had chronic or recurrent pyelonephritis and had previously undergone one form of antibacterial therapy or another, the results of the cultures do not necessarily reflect the initial etiologic agent of the urinary tract infection. The type and number of the microorganisms isolated in these cultures are graphically recorded in Figure 3. These data were compiled from all positive cultures regardless of the method of collecting the urine and the method of counting In addition to the microorganisms listed on this chart, a small number of miscellaneous species were isolated in a few instances, such as *Alkaligenes faecalis*, paracolon, and achromobacter. If one arbitrarily takes the colony count of 10,000 organisms per milliliter or greater as indicative of significant urinary tract infection, it becomes obvious that the gram-negative bacilli are the most common offenders. *Escherichia coli* was isolated most frequently (55 cultures), *Proteus* species next (29 cultures), and *Aerobacter aerogenes* and *Pseudomonas aeruginosa* least frequently (16 cultures each). There were fewer cultures revealing gram-positive organisms in large numbers, but enterococci were isolated in 14 and staphylococci in 9 Diphtheroids and monilia were occasionally isolated in numbers greater than 10,000 organisms per milliliter, but in no instance was there clinical evidence of pyelonephritis present when either monilia or diphtheroids were found. In one patient with 1,000,000 colonies per milliliter of monilia, a needle biopsy of both kidneys revealed no evidence of pyelonephritis The significance of the high counts of the other patients is difficult to appraise, but at least some of the high counts appeared to reflect an infection of the lower urinary tract.

In those cultures containing less than 10,000 bacteria per milliliter, the gram-positive organisms were isolated most frequently. Most of these undoubtedly represent contaminants or organisms present in the urethra and periurethral glands since many were present in concentrations of less

than 10 per milliliter and the cultures of specimens taken by aspiration of the bladder of these same patients were usually negative. On the other hand, some of the low counts were isolated from patients with clinical evidence of active pyelonephritis, and in three instances in which the counts were less than 1000 bacteria per milliliter the renal biopsy indicated

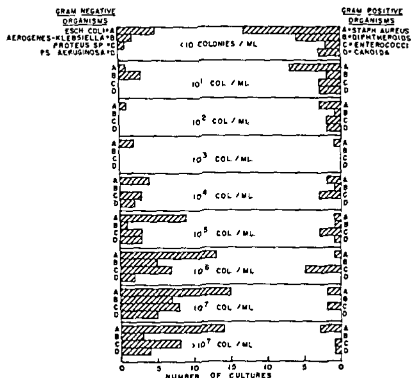


FIGURE 3 The results of 239 quantitative urine cultures on 121 patients.

definite changes of active pyelonephritis. The significance of the low counts in the others is unknown. Gram-negative bacilli were also occasionally cultured in small numbers, and attempts to correlate these results with other laboratory and clinical findings suggest that these too were mostly contaminants or organisms present in the lower urinary tract.

No attempt was made in this study to correlate in detail the cultural findings with the clinical diagnosis of pyelonephritis, since this has previously been done[3] and since the purpose of this study was to

us to provide the most significant information with the least effort, although there were false negatives in the 300 colonies per milliliter range.

Culturing the urine specimens in the thioglycollate broth almost always revealed growth when the urine was obtained by the midstream voided technique. The overgrowth of contaminants was so great that this method of culturing was considered worthless unless the specimen was obtained by bladder aspiration or careful catheterization.

Percutaneous needle biopsy of the kidney was carried out by Dr E. M. Ory one or more times in 51 patients who had clinical evidence of pyelonephritis or who had biopsies for some other reason and were found to have histologic evidence of pyelonephritis. Both kidneys were biopsied in six patients and one patient had a congenital absence of one kidney. More than one biopsy of the same kidney was performed in five patients.

Since many of the patients studied had chronic or recurrent pyelonephritis and had previously undergone one form of antibacterial therapy or another, the results of the cultures do not necessarily reflect the initial etiologic agent of the urinary tract infection. The type and number of the microorganisms isolated in these cultures are graphically recorded in Figure 3. These data were compiled from all positive cultures regardless of the method of collecting the urine and the method of counting. In addition to the microorganisms listed on this chart, a small number of miscellaneous species were isolated in a few instances, such as *Alkaligenes faecalis*, paracolon, and achromobacter. If one arbitrarily takes the colony count of 10,000 organisms per milliliter or greater as indicative of significant urinary tract infection, it becomes obvious that the gram-negative bacilli are the most common offenders. *Escherichia coli* was isolated most frequently (55 cultures), *Proteus* species next (29 cultures), and *Aerobacter aerogenes* and *Pseudomonas aeruginosa* least frequently (16 cultures each). There were fewer cultures revealing gram-positive organisms in large numbers, but enterococci were isolated in 14 and staphylococci in 9. Diphtheroids and monilia were occasionally isolated in numbers greater than 10,000 organisms per milliliter, but in no instance was there clinical evidence of pyelonephritis present when either monilia or diphtheroids were found In one patient with 1,000,000 colonies per milliliter of monilia, a needle biopsy of both kidneys revealed no evidence of pyelonephritis. The significance of the high counts of the other patients is difficult to appraise, but at least some of the high counts appeared to reflect an infection of the lower urinary tract.

In those cultures containing less than 10,000 bacteria per milliliter, the gram-positive organisms were isolated most frequently. Most of these undoubtedly represent contaminants or organisms present in the urethra and periurethral glands since many were present in concentrations of less

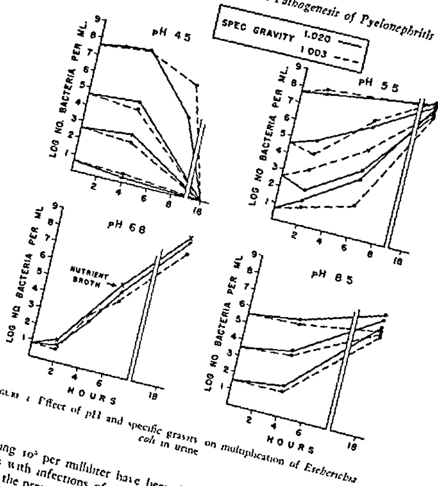


FIGURE 1. Effect of pH and specific gravity on multiplication of *Escherichia coli* in urine.

exceeding 10^5 per milliliter have been found in the urines of untreated patients with infections of the urinary tract. Since high colony counts indicate the presence of true bacteriuria, which is defined as the residence of bacteria within the urinary tract, it might be possible to separate bacteriuria from the contamination that occurs during the collection process. Such a separation would be possible if the number of contaminating bacteria were significantly less than the numbers characteristic of bacteriuria. A test of this view requires the study of a population group that is considered to be free of infection of the urinary tract. When the urines of 335 randomly selected female patients in the medical outpatient department of the Boston City Hospital were studied, excluding those patients who were thought by the house staff to have active infec-

Therefore, methods were needed for the detection of the relatively asymptomatic or clinically inapparent forms of infection of the urinary tract. Time does not permit a detailed exposition of all the methods of attack on this problem. Suffice it to say that the presence of pyuria is not an essential feature of the diagnosis in the way pyuria is usually sought, although it is probable that detailed studies of pyuria, using semiquantitative methods, may be more revealing than the casual search for pyuria. The finding of white cell casts does not distinguish between active disease and healed inactive pyelonephritis. The presence of pale-staining polymorphonuclear leukocytes in the urinary sediment has not proved to be a reliable index of the presence of active infection of the urinary tract.

However, by quantitative study of the bacterial flora of freshly obtained urine it has been possible to detect a large reservoir of asymptomatic infection of the urinary tract. The background for the quantitative approach will be summarized briefly.

Louis Pasteur first published in 1862, in the course of his experiments on spontaneous generation, the observations that urine is normally sterile and that urine is an excellent culture medium for many microorganisms.[20] Thus, in Figure 1, which depicts studies performed in collaboration with Dr. T. W. Mou, it is seen that if small numbers of coliform organisms are introduced into the urine they tend to multiply to numbers approximating those found in ordinary nutrient broth. Under ordinary physiologic circumstances, the degree of dilution and the changes in pH do not greatly alter the capacity of urine to function as a good culture medium for relatively nonfastidious organisms such as *Escherichia coli*. Only under exceptional circumstances is urine unable to support multiplication of the relatively nonfastidious organisms that occur in infections of the urinary tract.[6, 15, 22]

Clinicians have recognized for over fifty years that acute pyelonephritis is almost always accompanied by the presence of bacteria in stained smears of the urine. Since about 100,000 bacteria per milliliter of medium are necessary before an ordinary smear shows the presence of bacteria under ordinary conditions of oil immersion microscopy,[9, 11] it follows that the urine usually contains more than 100,000 bacteria per milliliter in most infections of the urinary tract. This is illustrated in Figure 2, which shows that 95 per cent of urines from consecutive patients who were diagnosed by the house staff as having pyelonephritis (without any additional analysis by the visiting staff of the adequacy of the diagnosis) contained more than 10^5 bacteria per milliliter. In many laboratories that have studied antibacterial agents during the past twenty to twenty-five years, it has been the practice to follow the progress of treatment using colony counts of serial dilutions of urine, and almost invariably numbers of bacteria

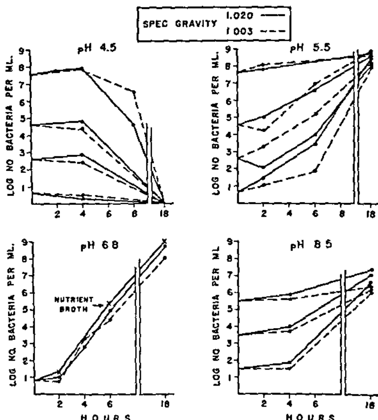


FIGURE 1 Effect of pH and specific gravity on multiplication of *Escherichia coli* in urine

exceeding 10⁵ per milliliter have been found in the urines of untreated patients with infections of the urinary tract. Since high colony counts indicate the presence of true bacteriuria, which is defined as the residence of bacteria within the urinary tract, it might be possible to separate bacteriuria from the contamination that occurs during the collection process. Such a separation would be possible if the number of contaminating bacteria were significantly less than the numbers characteristic of bacteriuria. A test of this view requires the study of a population group that is considered to be free of infection of the urinary tract. When the urines of 335 randomly selected female patients in the medical outpatient department of the Boston City Hospital were studied, excluding those patients who were thought by the house staff to have active infec-

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and epidemiologic purposes. The probability of error in accepting the results of low bacterial counts is extremely small and the error does not begin to become substantial until the range of 10^4 to 10^5 bacteria is encountered. In this range were found only 1 per cent of urines of individuals who had been catheterized. Half of these on second count were above 10^5 and half were well below this figure. That is, only rarely will counts in the doubtful range of 10^4 to 10^5 be encountered with catheterization, but when they are encountered the chances are approximately even that the patient is bacteriuric. On the other hand about 20 per cent of those presenting with voided specimens have counts in this range and, from comparison with the data from catheterized patients, only about one in 20 patients in this group can be expected to have true bacteriuria. This is confirmed by subsequent study of the urines of the patients who have been found to have 10^4 to 10^5 bacteria in voided specimens. The data may be summarized by the statement that the finding of 10^5 or more bacteria has a confidence level of about 95 per cent with catheterized specimens and about 80 per cent with voided specimens. Thus, two voided specimens bring the confidence to approximately 95 per cent and the confidence level can be increased still further by adding more specimens. It should be stressed that the consideration of bacteriuria versus nonbacteriuria is a statistical one in the absence of clear clinical symptomatology. There is no clear dividing line, but it is apparent from the data that 10^5 colonies per milliliter of urine may be taken to indicate bacteriuria for epidemiologic purposes, if two specimens agree on this range. All of the data that we have presented on the incidence of bacteriuria in population groups have been based on at least two specimens from bacteriuric individuals. On the other hand, in many clinical situations, it is necessary to accept the findings of a single specimen and the judgment of the clinician must take into account the statistical considerations, briefly considered here. The situation is not unlike that encountered when an individual patient presents with a fasting blood sugar 120 mg per cent. Yet for epidemiologic purposes the acceptance of an arbitrary line of demarcation between hyperglycemic and nonhyperglycemic individuals has some validity.

To demonstrate the validity of this level of bacterial count as evidence of bacteriuria it was necessary also to determine whether bacteriuria and pyelonephritis were related to one another. First it was argued that bacteriuria should occur more frequently in groups with increased incidence of clinical pyelonephritis. This is shown to be the case in Table III, which indicates that an increased incidence of bacteriuria occurs in those groups that may be anticipated, on clinical grounds, to have an increased incidence of pyelonephritis (for example, diabetic women, women with cystocele, patients with inlying catheters, pregnant women).

or with any other special circumstances However, in situations in which the results obtained from bacteriologic study are doubtful, it may be helpful to utilize the first morning specimen of urine since the bacterial counts tend to be highest when incubation in the urinary tract has proceeded for the longest possible period of time.

The establishment of *an objective method for the detection* of bacteriuria permitted a number of derivations to be made. First, it permitted the virtual discarding of the catheter for bacteriologic study of the urine In our last 5000 patients we have catheterized twice for the purpose of bacteriologic study This does not mean that we do not use the catheter. It means that we rarely use the catheter for bacteriologic study of the urine

Advantages of the voided specimen over the catheterized are obvious. The disadvantages must be stated. First, it is uncommon to obtain sterile urine by the voiding technique, therefore quantitative study of the urine is mandatory. Second, it is uncommon to find less than 100 to 1000 bacteria per milliliter of urine in voided specimens because of the greater likelihood of contamination. Third, because of the higher amounts of contamination, more false positive results are to be anticipated To deal *with this problem*, it is essential that an adequate statistical analysis of the data be utilized. This is not an appropriate place in which to present a detailed analysis. Suffice it to say that if one uses as a simple approach to the problem the comparison of two bacterial counts of urine obtained from the same patient at different times, one can arrive at a statement of the likelihood that a second count will agree with the first in terms of the presence or absence of bacteriuria. This information is depicted in Table II. It can be seen that the margin of error that is introduced by the use of voiding techniques is greater but far from prohibitive for clinical

TABLE II. DISTRIBUTION OF BACTERIAL COUNTS OF URINE IN SUCCESSIVE VOIDED AND CATHETERIZED SPECIMENS

Bacterial Count	Per Cent of Total		Per Cent Probability That Second Count Contains $>10^5$ Gram-negative Rods	
	Catheterized	Voided	Catheterized	Voided
0	46	2	<1	<1
10^{0-1}	22	9	<1	<1
10^{1-2}	15	7	<1	<1
10^{2-3}	7	23	<1	<1
10^{3-4}	4	32	2	2
10^{4-5}	1	19	50	5
10^{5-6}	2	5	90	67
$>10^6$	4	3	98	95

pyelonephritis occurred in individuals with no bacteria in their urine. However, of these three, two were patients who had had bacteriuria during their last hospital admission and had been treated with antibacterial agents. The one patient who was an exception was in the hospital for only twelve hours before death, had been operated on in another state for pelvic carcinomatosis, and had presumably been extensively treated there, but we have been unable to obtain a full record of her previous illness.

The autopsy studies therefore indicated that there was an association between active pyelonephritis and bacteriuria but, as Table IV shows, there was no association between bacteriuria and healed pyelonephritis.

It may be useful to stress again the need to distinguish active healed pyelonephritis in further studies of the relationships of bacteriuria and pyelonephritis to chronic renal disease and hypertension. We have restricted the term "active pyelonephritis" to situations in which chronic inflammatory changes associated with lymphoid and plasma cell infiltrations may not be present but in which polymorphonuclear foci in the interstitial areas of the kidneys are present. This infection is based on the studies of Weiss and Parker,[21] who pointed out that chronic pyelonephritis could be divided into active infections showing foci of acute inflammation in the interstitium and healed lesions. Healed pyelonephritis has received much discussion and is a major problem about which we know very little. Whether it is regularly related to active pyelonephritis remains to be demonstrated. On the other hand active pyelonephritis, whether acute or chronic, seems clearly to be related to infection of the urinary tract and, as I hope we have demonstrated, to be related to bacteriuria. From the data presented it would be useful if future studies of pyelonephritis included data on the state of bacteriuria of the patient and, where possible, data on the nature of the chronic pyelonephritis with respect to the number of polymorphonuclear cells in the interstitium. In this way it may be possible to separate parts of the problem from the entire problem and thus make some progress.

To return to the central thesis, the observations thus far presented indicate a relationship between bacteriuria and pyelonephritis but do not tell us which came first or what the pathogenetic role of bacteriuria might be.

About three-quarters of the bacteriuric pregnant women became free of bacteriuria with 0.5 Gm. of sulfamethoxypyridazine daily. These were primarily the patients with *E. coli* bacteriuria. About one-fourth of the patients, most of whom were infected with *A. aerogenes*, were treated with nitrofurantoin. In only one instance, thus far, has it been necessary to use a third drug based on *in vitro* sensitivity studies. When we speak of a patient as having been treated, we mean that the bacteriuria was elimi-

TABLE III. OCCURRENCE OF BACTERIURIA IN VARIOUS POPULATION GROUPS SURVEYED AT BOSTON CITY HOSPITAL

[illegible]
[illegible]
[illegible]
5% of 37 diabetic males in outpatient department
[illegible]
who underwent single catheterization
[illegible]

In addition, the table indicates the magnitude of the incidence of bacteriuria in various population groups, and charts out the possibilities of an attack on this problem as a major problem in preventive medicine.

The relationship of bacteriuria to pyelonephritis was explored at autopsy.[13] Urine was aspirated from the bladders at the beginning of 100 random autopsies and bacterial counts determined. Independently, the kidneys were removed and studied by two pathologists who were not aware of the bacteriologic findings and who made their observations independently of each other. When the three sets of data, the bacteriologic and two sets of morphologic data, were put together, there were only minimal disagreements between the two morphologists. The data are shown in Table IV. It should be pointed out that 53 per cent of the urines had no bacteria per milliliter of urine and only 7 per cent had between 1 and 10,000 bacteria. The remaining 40 per cent had more than 1,000,000 per milliliter of urine. Of the 40 per cent with bacteriuria, 14 autopsies had active pyelonephritis. Three instances of active

TABLE IV. PATHOLOGICAL LESIONS OF THE URINARY TRACT IN RELATION TO BACTERIAL COUNTS OF THE URINE AT AUTOPSY

	Bacterial Counts per Milliliter of Urine	
Lesion	$>10^5$ (40 patients)	$<10^5$ (60 patients)
Acute or chronic pyelonephritis	14	3
Focal or diffuse healed pyelonephritis	5 (1)	13 (1)
Acute pyelitis	2 (2)	0
Chronic pyelitis	1	0
Acute cystitis	6 (3)	0
Chronic cystitis	1 (1)	1

These women are being followed over an extended period of time in order to determine what their clinical course will be.

When the fate of the infants that were born to these patients was studied, it was found that there was an unusually high infant mortality in untreated bacteriuric mothers, and not in the treated women. The cause of death in the infants who had come to autopsy was not sepsis, and mothers who had delivered these babies had no clinical evidence of sepsis. The cause seemed to be prematurity, hyaline membrane disease, atelectasis, and other findings associated with prematurity. Therefore, the incidence of prematurity in the entire group was investigated and it was found that the incidence of prematurity in untreated bacteriuric women was 24 per cent, whereas in treated women and in 1000 consecutive nonbacteriuric women the incidence of prematurity was 9 to 10 per cent (Table VII). Even treatment late in pregnancy, given in association with appearance of clinical symptoms of infection, was sufficient to protect the mother and child against prematurity. If these data are borne out in forward studies that are now in progress it may be possible to prevent 10 to 20 per cent of prematurity and perhaps as much as one-third of neonatal death, simply by detecting and treating bacteriuria early in pregnancy.

TABLE VII Occurrence of Prematurity and Infant Death in Relation to Bacteriuria During Pregnancy

Patient Group	Neonatal Deaths	Premature Infants
Control	17%	24%
Treated	0	10%
Nonbacteriuric	3%	9%

Many additional findings in pregnant women must cause us to evaluate the problem of pathogenesis of pyelonephritis with greater care. The incidence of bacteriuria in pregnancy was plotted in relation to the duration of pregnancy at the time of the first prenatal visit (Figure 3). It is seen that most, if not all, bacteriuria is acquired before the second month of pregnancy. The increased incidence of bacteriuria after the second month of pregnancy is not yet statistically significant but may be real. In any event, it is not great. This wholly unexpected finding indicates that bacteriuria has appeared before the anatomic changes of pregnancy have had time to appear. Obviously, the anatomic changes of pregnancy become progressively more marked as pregnancy proceeds, and yet the incidence of bacteriuria does not rise appreciably. It may therefore be argued that the anatomic changes in pregnancy may relate

nated promptly and remained absent until the time of delivery. In earlier experiences, treatment was stopped while the patient was still pregnant and bacteriuria tended to recur.

Alternate patients were given a placebo. The patients were followed in the obstetrical clinic as well as in the treatment clinic, and the obstetricians were not aware of the nature of the treatment given to the patients under study.

Table V shows that about 40 per cent of untreated pregnant women with bacteriuria will develop pyelonephritis of pregnancy. Most of

TABLE V. Effect of Treatment on Occurrence of Pyelonephritis in Prenatal Patients with Asymptomatic Bacteriuria

Patient Group	Number of Patients	Number with Pyelonephritis	Per Cent with Pyelonephritis
Placebo	48	20	42
Treated*	43	0	0

* Only patients who were freed of bacteriuria and were under continuous treatment until delivery are included

them developed clinical pyelonephritis during the latter half of pregnancy or shortly after delivery. So far, no woman whose bacteriuria was eliminated throughout pregnancy has developed pyelonephritis of pregnancy, and no woman who did not have bacteriuria at the time of the initial screening of the urine has developed pyelonephritis of pregnancy. Thus, pyelonephritis of pregnancy is apparently more or less completely

TABLE VI. Incidence of Bacteriuria 3 to 12 Months Post-partum in Patients with Bacteriuria During Pregnancy

Patient Group	Bacteriuria Present	Bacteriuria Absent
Placebo	15*	4
Treated	3	14

* Three of these patients developed acute pyelonephritis after three-month follow up They were treated and were free of bacteriuria in the post-treatment cultures

preventable and bacteriuria has predictive value in delineating the group of patients out of which will come those who will develop pyelonephritis

The untreated asymptomatic bacteriuric women were studied three and twelve months after delivery (Table VI). The untreated women retained their bacteriuria in more than three-quarters of instances, whereas the treated women regained bacteriuria in less than one-quarter of cases.

GENERAL DISCUSSION

DR RELMAN. I should like to say something about the question of so called "inactive" or "healed" versus "active" chronic pyelonephritis.

I would agree with Dr. Kass that it is well for us to be precise in the terminology we use to describe the histology of chronic pyelonephritis, and it is certainly true that many pathologists rely on the presence of polymorphonuclear leukocytes as an indication of whether or not a given case of chronic pyelonephritis should be called "active" But I am not at all sure that this definition of activity correlates well with *clinical* activity, in terms of progression of renal damage and development of hypertension Therefore, it seems to me that in considering the question of the relationship of bacteriuria to chronic pyelonephritis we should not restrict ourselves by applying an arbitrary histologic criterion of activity unless it can be shown that this criterion is in fact meaningful in terms of the clinical behavior of the disease.

Using the criterion of polymorphonuclear leukocytes in the tissues, I am not sure whether all our cases of chronic pyelonephritis would be called "active," although some certainly did have polymorphs However, all of them showed an extensive interstitial inflammatory reaction with plasma cells and lymphocytes, and the great majority had evidence of progressing renal disease or hypertension or both To use the term "inactive" or "healed" in such cases, if one does not find polymorphs, seems to me to be misleading

Finally, I wish to say that our preliminary experience with the treatment of chronic pyelonephritis in pregnancy entirely supports Dr. Kass's observations Our impression is that when women who have had recurrent episodes of pyelonephritis and toxemia of pregnancy in the past are given continuous suppressive antibacterial therapy from the beginning of their current pregnancy, one sees a much improved result. Suppression of bacteriuria during pregnancy appears to prevent acute flare-ups of pyelonephritis and may have something to do with preventing recurrence of toxemia

DR AUSTRIAN I should like to return to the esoteric aspects of bacteriology for a moment In addition to the metabolic classification brought out by Dr Edwards and to the important serological methods referred to by Dr. Neter, there have been some recent interesting developments relating to the genetics of the enteric bacteria.

Doty and his group at Harvard have shown that the purine and pyrimidine base composition of the genetic determinants of *Escherichia coli* and *Aerobacter aerogenes* are distinctly different, which fact may

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progress Any time the infection flared up and we had an increase to 100 million and more of leukocytes, there was a tendency f glomerular filtration to decrease.

The second point concerns the name Should we use the name "healed pyelonephritis? Healed pyelonephritis is actually a condition with con tracted scarred kidneys and the patients may die of uremia I think we should drop a name that suggests that the lesion is healed

DR KASS Dr. Relman, no one who sees patients would argue that there is no relevance to separating very severe lesions from less severe lesions However, it is equally valid to argue that, on the basis of specific etiologic grounds and specific relationships of disease, we must separate disease states into as many relevant categories as possible

So there is no real disagreement except in terms of our objective at the moment My objective was to try to define the specific relationship between bacteriuria and pyelonephritis, and the only form of pyelonephritis with which I can make this relationship is active pyelonephritis This relationship does not negate the fact that there are many people with serious disease of the kidneys who don't have active pyelonephritis

With respect to Dr Davis's comments, I hope he has enjoyed as much as I have the correspondence we have had, which, as you have gathered, was quite spicy in spots We have exchanged views for some time, with not much change in our points of view Since we corresponded about Crabtree's book, Dr Davis knows that I have studied it carefully In that excellent book, Crabtree points out that there is such a thing as bacilluria, which is defined in his book as the obtaining of any kind of positive culture from a urine obtained by catheter He points out, as Dr. Davis and Dr Young also pointed out, that *Escherichia coli* is the commonest pathogen of the urinary tract, but that the next most common pathogens are staphylococci, with enterococci not far behind

Crabtree realized that there was no way that he could detect contamination and contrast it with true residence of bacteria within the urinary tract He makes clear that it was impossible for him to evaluate the meaning of what he calls bacilluria because he recognized that contamination plays a large role, and he was simply at a loss to separate contamination from bacteriuria

His figures for the incidence of bacteriuria are very much higher than our figures, as you might anticipate As has been shown adequately, and I think is now simply to be accepted, staphylococci and enterococci are uncommon pathogens of the urinary tract Most of the reports that list them in 25 to 30 per cent incidence give us a pretty accurate view of the extent of contamination when a single catheterized specimen is cultured in the usual way I certainly don't intend to slight any of the

earlier work. I hope that in the course of the twenty-odd years since Crabtree's work a few more facts have been added to the others.

With respect to the complicated problem of ureteral dilatation and the whole problem of structural abnormality of the urinary tract that Dr. Nesbit has raised, I don't know the answer. We did not look for radiologic abnormalities. Obviously this is a thing to be avoided in pregnancy.

However, I cannot feel that the anatomic changes are primary causes of bacteriuria. The incidence of bacteriuria does not rise with continued pregnancy and is about as great at two months as later. It could be argued that the appearance of acute symptoms is related to the progressive changes in the anatomy of the urinary tract that occur later in pregnancy, but I don't think it can be held that the anatomic changes of pregnancy lead to bacteriuria.

I think what we are doing once again is falling into the problem of confusing bacteriuria with pyelonephritis in the clinical sense of the word. I was referring only to bacteriuria. Pyelonephritis in the clinical sense of the word occurs in only 40 per cent of these patients, and I think a different sequence of events is necessary to explain how the bacteriuria becomes clinical pyelonephritis than to explain how bacteriuria occurs.

With respect to the problem of fever and prematurity, and so on, it is well documented in the history of infectious diseases that patients with severe infections tend to have precipitous deliveries. It is documented that patients with severe pyelonephritis have a high incidence of prematurity.

The only point I was making here is that our patients did not have fever and did not have clinical illness, and still they had a high incidence of prematurity. It seems to me this is a different dimension from that which has usually been held. We are dealing with asymptomatic and unsuspected infections that lead to widespread physiologic disturbances.

I have no answer to Dr. Shapiro's question concerning the incidence of toxemia. It was quite low, and it is too soon for us to be able to answer.

29

Natural Inhibitors of Bacterial Multiplication

DERRICK ROWLEY, M.D., Ph.D
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Although it is a commonplace, it is probably worth restating that the outcome of bacterial infections is the resultant of the two opposing tendencies, bacterial multiplication on the one hand and the destruction of bacteria by various host mechanisms on the other. The more chronic the infection, the more closely is a balance maintained between the two tendencies. A very slight change in the rate of either of these may upset the balance and terminate the conflict one way or the other. There are numerous examples which illustrate this delicate balance.

Some streptomycin-resistant variants of *Salmonella typhimurium* have been found to be considerably less virulent for mice than the parent strain,[1] and this was associated with slight increases in the mean generation time of the mutants *in vitro*. Similarly, the resistance of rabbits to some strains of pneumococci has been correlated with slower growth rates of those strains at the relatively high temperature of the rabbit.[2] Indeed, the results of the classical experiment of Pasteur in which chicken became susceptible to anthrax after standing in cold water may well have been due to a slight fall in body temperature which provided the bacteria with a more favorable growth rate. If such small changes in environmental temperature can influence disease processes, it is an intriguing speculation whether pyrexia may not often be a response of benefit to the host.

Total body irradiation of animals is often followed by invasion of gram-negative bacteria which almost certainly originate from the normal flora of the gut. From this and other findings there is no doubt that the healthy animal body is resisting bacterial penetration all the time. This process is usually "silent" since the host rapidly eliminates most of these organisms, very occasionally such microbes grow almost as rapidly in their host as they do under test-tube conditions and then the fatal attack is described as "fulminating." Less rarely the two opposing tendencies of bacterial growth and destruction take time to be resolved and are accompanied by signs and symptoms of disease for a variable period. It is clear that most infections which reach doctors are quite delicately balanced states, and this seems to be particularly true of kidney infections.

they act most efficiently together.[3, 8] Recent results demonstrate that in some situations the supply of these opsonic factors may be limiting to the whole process of cellular bactericidal activity. Benacerraf and co-workers[1] have shown that the rates of clearance of bacteria by the intact reticuloendothelial system are related to the antibody levels in the plasma Our own studies on intraperitoneal infections of mice demonstrate that in the normal mouse peritoneum there is little or no uptake and removal

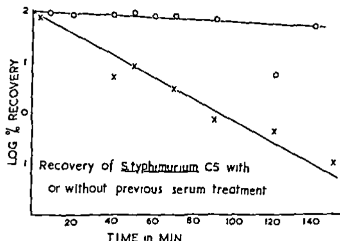


FIGURE 2. Recovery of *Salmonella typhimurium* C5 from the mouse peritoneum X – X, organisms previously treated with antiserum, O – O, untreated organisms

of gram-negative bacteria by macrophages in the course of the first hour or two (Figure 2) By contrast the same strains of bacteria which have been presensitized by antibody are engulfed and killed by the peritoneal cells with extraordinary speed. The same rapid rate of removal is seen when unsensitized *S typhimurium* is injected intraperitoneally into immunized mice, thus demonstrating that failure to remove bacteria in normal mice is due to lack of antibody and not to local restricted transport of such antibodies to the peritoneum.[14]

Mouse macrophages can effectively kill many species of gram-negative bacteria at similar rates once they have been phagocytosed. On the other hand there are great differences in the rates of phagocytosis of virulent as compared with avirulent bacteria, in the presence of mouse serum (Figure 3). The rate of this uptake process is entirely dependent on the concentration of antibody – up to a maximum – and it seems possible that a contributory reason for the virulence of most *S typhimurium* strains for mice is to be found in the shortage of antibody or opsonic factors in the serum and tissue fluids of mice (Figure 4). Conversely,

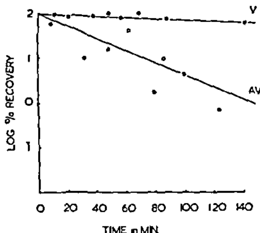


FIGURE 3 Recovery of virulent (V) and avirulent (AV) strains of *Salmonella typhimurium* from the mouse peritoneum

other animals and humans might owe their resistance to invasion by these organisms to a sufficiency of opsonins allowing rapid phagocytosis and digestion of the bacteria.

Our findings may be briefly summarized as follows Resistance to intraperitoneal infections in mice is largely determined by the rate at

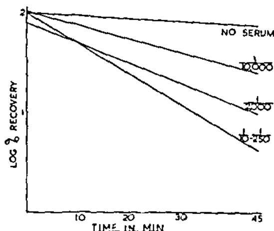


FIGURE 4 Effect of pretreatment with dilutions of specific antiserum (agglutination titer 1/100) on the recovery of *Escherichia coli* 2206 from the mouse peritoneum.

which the organisms can be taken up and destroyed by the mononuclear cells which abound in the normal mouse peritoneum. The vital rate-determining step is the act of phagocytosis, which is entirely controlled by the supply of humoral opsonic factors We have attempted to test the *in vivo* importance of these ideas by subjecting *S. typhimurium* organisms to heat-inactivated serum taken from various normal animals, for a few minutes before dilution and intraperitoneal injection into mice.[9] *This procedure, while not affecting the viability of the organisms in vitro,* was found with some sera to reduce greatly the lethality for mice of the injected bacteria. Moreover, this protective power could not be correlated with the content of antibody against the bacterial O somatic antigen (Table I). It seems likely that this effect is due to the presence in some normal sera of opsonic antibodies directed against components of the bacterial cell other than the O antigen

It is my hope that this short presentation will re-emphasize to you the impossibility of separating defense mechanisms into humoral and cellular types Even the highly effective cellular mechanisms are limited ultimately by the supply of humoral factors.

How may these considerations apply to the present study of kidney infections? Earlier in this paper, consideration was given to the delicate balance which exists in most infectious states between the processes of the host tending toward recovery and those of bacterial growth. One paramount feature stands out in the pathogenesis of pyelonephritis, namely, the observation that obstruction of urinary flow renders the organ susceptible to infection It seems probable that this circumstance upsets the almost symbiotic host-bacterial relationship by reducing the

TABLE I EFFECT OF PRETREATING *Salmonella typhimurium* WITH SERA OF NORMAL ANIMALS ON ITS LETHALITY FOR MICE

Serum	Specific Hemagglutination Titer of Serum*	28-Day Mortality with 15 Mice
Horse	160	2
Human	80	10
Chick	80	12
Pig	8	1
Rat	2	4
Mouse	0	15
Guinea pig	0	15
S paratyph B rabbit antisera	80	8
Controls (no serum)	—	15

* This was determined by coating sheep red cells with the specific lipopolysaccharide extracted from *S typhimurium* C_5, and titrating against the serum.

supply of serum factors, which are vital requirements for antibacterial activity in either of the two well-established defense reactions Whether this reduced availability is due to a decreased blood flow or to back pressure effects or to some other physical or mechanical defect is not yet clear. On the other hand the rapid recovery which often accompanies the relief of pressure in infected areas, without any other treatment, is further testimony to the small quantitative differences in rate processes which determine recovery or progressive infection

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*The Role of Natural Inhibitors in the Mechanisms of Localization of Bacteria in the Kidney**

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In the large amount of study which has been devoted to the pathogenesis of infections, surprisingly little attention has been paid to the individual susceptibilities of certain organs. Instead the tendency has been to concentrate on general factors operative in all tissues — for example, antibodies, phagocytic cells, and nutritional, hormonal or genetic influences. It would appear that more consideration might profitably be given to such questions as why the pneumococcus does not cause pyelonephritis, or the meningococcus pneumonia.

The subject of this symposium presents a nice example of the peculiar susceptibility of one organ to infection by one group of bacteria. The great majority of cases of acute pyelonephritis are caused by members of the coliform family. No particular type seems to be responsible, those isolated from patients with urinary tract infection comprise a heterogeneous group.

From the clinical standpoint, what do we know about infections by these organisms? Young infants may suffer such processes as enteritis, omphalitis, or meningitis due to coliform bacterial infection. In later life these organisms may also be found among the mixed flora of infections arising by direct spread from the intestinal tract — for example peritonitis and appendicitis — but here they are not acting alone, rather, they are participating in company with various other organisms, especially anaerobic bacteria. With those exceptions the impressive fact is this: pure-culture infections by *Escherichia coli* are mainly encountered in the urinary tract. It would seem, then, that these bacteria, which exhibit a low order of virulence for most tissues of the human body, find it comparatively easy to bring about an infection of the kidney.

Experiments with laboratory animals confirm these clinical observations. Rabbits do not succumb to intravenous inoculation of large numbers

*Experimental work mentioned in this paper was carried out at the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, England.

unless the inoculum is from an aging culture containing comparatively large quantities of endotoxin.[11] Mice infected by the intraperitoneal route do not die unless mucin is incorporated in the inoculum.[12] We have observed the effect of direct injection of large numbers of organisms into such tissues as skin, subcutaneous tissue, muscle, liver, spleen, and lung. The usual result is a mild inflammatory reaction in the area, from which living bacteria quickly disappear. In the case of the kidney, however, an acute suppurative process can be produced by injection of as few as 10 to 100 living bacteria into the medullary zone[2] Furthermore, in normal rats which have been given an intravenous injection of a culture of *E. coli*, those bacteria which happen to be trapped in the kidney seem to be destroyed less efficiently than the ones trapped in other organs such as lung, liver, and spleen.[4]

There is evidence then, both clinical and experimental, that *E. coli* finds the kidney a more favorable environment than other tissues of the body. Two possibilities suggest themselves. Firstly, something in the kidney may facilitate growth of the bacteria. This seems unlikely, because the coliforms are not fastidious, and grow rapidly on most infusion media. Secondly, something in the kidney may interfere with natural mechanisms for dealing with the bacteria. This is the possibility we have been examining

It is well known that gram-negative bacilli can be killed readily by body fluids which contain antibody and complement. Heat-stable proteins capable of reaction with coliform bacteria (natural antibodies?) can be demonstrated in the serums of most human beings and animals beyond the neonatal period of life. In view of the ease with which coliform bacteria are eliminated from most tissues of the intact animal there is reason for the assumption that this kind of bactericidal mechanism is partly or wholly responsible for natural resistance against gram-negative bacillary infection

The work we are reporting[1] began with a series of experiments to test the possibility that there is something in kidney tissue which interferes with the bactericidal action of normal serum. Such an effect was immediately and easily demonstrable An example is shown in Figure 1, where the effect of homogenates of kidney and liver from the same animal are compared, in a serum bactericidal system. This shows that the bacteria are destroyed almost as rapidly in the presence of liver tissue as in its absence, whereas kidney tissue seems to protect bacteria from destruction by serum

The mechanism of this effect could be an action on complement, antibody, or on the bacteria. By employing a system involving lysis of sensitized erythrocytes it was found that the effect of kidney tissue homogenate is on complement. Kidney tissue possesses five to fifteen times as

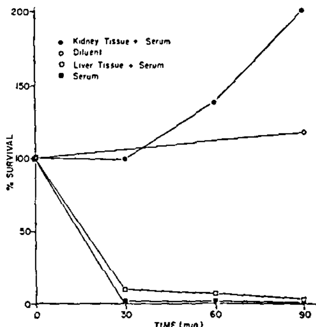


FIGURE 1 Bactericidal action of normal serum for *Escherichia coli* in the presence of homogenates of normal organs

much anticomplementary action as other tissues, when compared on a basis of dry weights (Table I)

Efforts were then made to learn something about the nature of the anticomplementary agent in kidney tissue It appeared to be associated with tissue particles, since the active ingredient could be sedimented by ordinary centrifugation It was destroyed by heating at 60° C., and was

TABLE I THE ANTICOMPLEMENTARY EFFECT OF HOMOGENATES FROM VARIOUS RABBIT ORGANS

Tube No	1	2	3	4	5	6
Per cent Hemolysis with						
Kidney	0	0	10	50	70	90
Spleen	70	90	100	100	100	100
Liver	60	90	100	100	100	100
Lung	60	90	100	100	100	100
Heart	80	100	100	100	100	100

Note Each homogenate had been adjusted to the same salt-free dry weight, and the tube numbers indicate successive twofold dilutions of homogenate

not dialyzable. Efforts to separate it from particles and obtain it in soluble form met with little success, because the active substance was easily destroyed by physical and chemical treatments For example, about 50 per cent of the activity disappeared during 2 minutes of treatment by ultrasonic vibrations. Repeated freezing and thawing also caused considerable loss in activity, as did freeze-drying Pieces of whole kidney, on the other hand, could be stored for months in the frozen state without significant deterioration. Gradual loss was noted when suspensions of homogenized tissue were maintained at 4° C. for several days.

Study of the effect of temperature on complement inactivation by kidney tissue showed that there was marked acceleration in rate of the reaction as the temperature increased from 20° to 40° C. This finding is more in keeping with a chemical reaction than a physical adsorption

To learn which component was affected by kidney tissue, tests were made, employing serum reagents lacking one or more of the four known components. The results indicated that the effect is mainly on the fourth component This finding directed our attention to the possibility that the mechanism of the kidney's anticomplementary effect involved production of ammonia, since the fourth component was discovered by virtue of its susceptibility to injury by ammonia.[3]

The ammonia formed in the kidney is largely, or wholly, derived from glutamine[14] This process is activated in the presence of acidosis. The enzyme responsible for the reaction, called glutaminase I, has not been isolated in pure state. Its distinctive characteristic, in addition to capacity to liberate ammonia from glutamine, is enhancement of activity in the presence of phosphate[10]

We carried out a series of experiments testing for similarities in characteristics of the anticomplementary effect of kidney tissue and in glutaminase activity. The results showed that there were parallels between these two functions The quantity of ammonia liberated during incubation of kidney homogenate appears to be adequate to account for the complement inactivation that had been observed. The most decisive findings were those in tests of the effect of glutamine and phosphate on the anticomplementary system Both agents augmented the effect. It can be concluded with reasonable assurance that kidney tissue interferes with the lysis of bacteria *in vitro* by inactivation of the fourth component of complement, and that the mechanism is the formation of ammonia.

How can this work be related to the pathogenesis of pyelonephritis? Obviously much remains to be done Little proof can be offered, beyond observations similar to Pfeiffer's finding of rapid destruction of cholera vibrios in the peritoneum of an immune animal, that complement participates in host defense mechanisms *in vivo* Nevertheless we have based

the present work on the concept that complement does have a function, and that with antibody it is one of the operative factors in the rapid destruction of gram-negative bacilli which takes place in most tissues of an immune animal

In the kidney, it is conceivable that a minor degree of complement inactivation goes on all of the time, this could have some bearing on the peculiar persistence of small numbers of bacteria there following intravenous injection.[4] In circumstances of greatly increased renal ammonia production, as in acidosis, complement inactivation might become sufficient in some parts of the kidney to permit bacterial growth and a true infection.

Certain items of clinical information seem to be in line with the possibility that alterations in acid-base equilibrium play a part in the pathogenesis of renal infections One thinks immediately of the fact that pyelonephritis is more common and more severe in patients with diabetes, who, because of acidosis, are likely to have increased ammonia production [9] Also of interest is the fact that alkali therapy has long been advocated in the treatment of acute pyelonephritis The medical literature previous to 1930 contains reports of dramatic subsidence of fever and other manifestations in "acute pyelitis" following institution of alkali treatment [3, 13] However, those clinical impressions could not be supported by bacteriologic tests, since alkali therapy did not diminish the bacterial content of the urine Consequently the appaarent clinical improvement following alkali therapy came to be regarded as mere coincidence, and that treatment fell into disuse, to be supplanted by the use of acidifying agents and mandelic acid, since these agents unquestionably affect the growth of bacteria in urine In the light of more recent findings, particularly Kass's demonstration of the frequency of bacteriuria,[x] there can be no question that heavy growth of bacteria in the urine frequently takes place independent of kidney infection One can conceive of alkali therapy exerting a beneficial effect on infection in the tissues of the kidney without corresponding improvement in results of urine cultures It is of interest also to recall that early experience with streptomycin in treatment of pyelonephritis showed that the results were better when alkalis were given with the antibiotic [7] This was ascribed to the greater effectiveness of streptomycin in urine at an alkaline pH, since alkali treatment would not greatly alter the pH of renal tissues.[x] Possibly the good results were due in part to the creation of conditions more favorable to host defense mechanisms in the substance of the kidney.

Tests of the relevance of this work to the pathogenesis of experimental pyelonephritis will be described in the paper which follows.

SUMMARY

In experiments undertaken to elucidate the vulnerability of the kidney to infection by *E coli* it was shown that kidney tissue is unique in its capacity to interfer with the killing of these bacteria by normal serum. The mechanism of the interference is an anticomplementary action, involving inactivation of the fourth component of complement, probably by the formation of ammonia. Since ammonia formation can be made to vary widely, depending on acid-base equilibrium, these findings suggest another means of influencing the susceptibility of the kidney to infection by gram-negative bacilli.

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*The Effect of an Acidifying Salt on the Susceptibility of the Kidney to Infection**

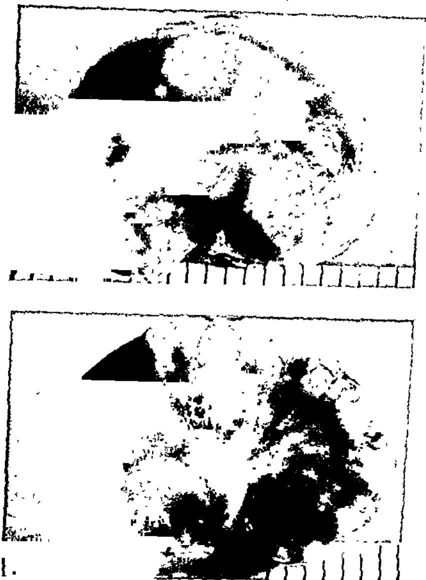
LAWRENCE R. FREEDMAN, M.D.
(New Haven, Connecticut)

The preceding paper described the *in vitro* anticomplementary effect of renal tissue and its probable dependence upon renal ammonia production. The present studies were undertaken to test in animals whether increasing the activity of the renal ammonia-forming mechanism would impair the process by which bacteria are normally cleared from the kidney. If such a relationship could be established, it would constitute an *in vivo* demonstration of the role of complement in the body's defenses against bacterial invasion. The studies to be reported appear to be compatible with the existence of such a mechanism.

It is well known[1] that administration of NH_4Cl is a potent stimulus to renal glutaminase resulting in increased NH_3 production. The general plan of the experiments to be described was to administer *Escherichia coli* intravenously to rats and then to compare the disposition of these organisms in animals drinking NH_4Cl with that of animals drinking suitable control substances. The finding of 10^5 or more bacteria in the quantitative culture of homogenates of whole kidney was taken to indicate infection. The details of the methods employed have been described in previous reports from this laboratory.[2] It should be emphasized that previous studies with the particular *E. coli* used in these experiments have demonstrated that this organism given intravenously does not produce infections in normal rats,[2] rats acutely depleted of potassium,[3] or rats poisoned with mercuric chloride.[4]

The initial experiment compared 12 rats drinking 2 per cent NH_4Cl with 6 rats drinking the same volume of tap water. The animals were pair fed. The standard inoculum of *E. coli* was administered to all animals 2 days after the start of the experiment, and the animals were sacrificed 7 days later. Cultures of lung tissue and blood gave no growth. Gross examination of the mediastinum, peritoneum, liver, and spleen did not reveal evidence of infection. Kidney infections were observed in over

* This work was supported by research grant E-1850(C) from the U.S. Public Health Service.



FIGURES 1 and 2 Examples of the gross abscesses produced by *Escherichia coli* in rats drinking 1.6 per cent NH_4Cl.

infection is related to a rise in *glutaminase activity, one would expect that* the incidence of infection would reflect the duration of acidifying treatment. Accordingly, 12 rats were begun on 1.6 per cent NH_4Cl drinking water 5 days prior to their receiving *E. coli*, whereas another group of 12 rats, drinking plain water and pair-fed with the first group, was started

on NH_4Cl only 1 day prior to the intravenous injection of *E. coli*. Both groups continued to drink NH_4Cl solution for an additional 3 days, at which time the kidneys were examined. In group A, which had been drinking NH_4Cl for a total of 8 days, 6 of 24 kidneys were infected. In group B, which had been on NH_4Cl for a total of 4 days, no infections

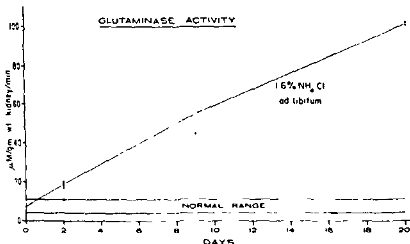


FIGURE 3 Glutaminase activity is indicated as micromoles of NH_3 produced per gram of kidney (wet weight) per minute

occurred It would appear, then, that the degree to which the rats are made susceptible to infection is correlated with the length of time of ingestion of NH_4Cl It seems likely that if larger numbers of animals had been used, some infections would have occurred in the group begun on NH_4Cl one day before the injection of *E coli* since it had already been found that even when NH_4Cl was begun after the bacteria were given, some renal susceptibility to infection had been induced.

The final experiment to be presented was designed to determine whether administration of alkali would reverse the effect of the acidifying salt on susceptibility. Fifty-four rats were given 1 6 per cent NH_4Cl to drink *ad libitum* for about 7 days. The half of this group showing the greatest weight loss was then allowed to drink varying amounts of 2 5 per cent (equimolar) sodium bicarbonate for from 3 to 24 hours, at which time all animals were inoculated intravenously with *E coli*, and the same drinking solutions continued Following the administration of these organisms, the fluid intake of the rats drinking $NaHCO_3$ was limited to that of the NH_4Cl group. From 5 to 7 days later, bacteriologic examinations of their

tissues and body fluids were carried out. The results are given in Table IV In the $NaHCO_3$ group, 3 of 54 kidneys were infected. Urine culture was positive in only 2 of these animals. In the group allowed to continue drinking NH_4Cl, 22 of 54 kidneys were infected, representing infections

TABLE IV. INFECTION PRODUCED BY 10^5 *Escherichia coli* GIVEN INTRAVENOUSLY TO RATS FED AMMONIUM CHLORIDE OR AMMONIUM CHLORIDE AND SODIUM BICARBONATE

	1 6% NH_4Cl	1 6% NH_4Cl 3–24 hours 2 5% $NaHCO_3$
Kidneys infected	22/54	3/54
Animals infected	13/27	3/27
Gross abscesses	6/13	0/3
Average weight loss at time of division	11 3%	18 1%

in 13 of the 27 animals. All of these infected animals had positive urine cultures. The blood cultures in both groups were negative. It can be concluded from this experiment that the mechanism by which NH_4Cl increases renal susceptibility to infection is largely nullified by the administration of an alkalinizing agent. These results suggest that this effect is a biochemical one.

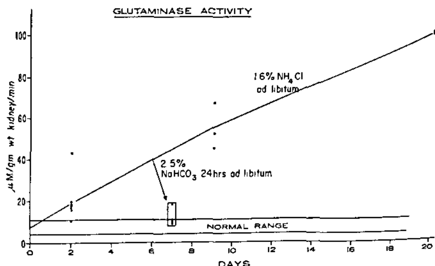


FIGURE 4 The arrow originates from the point on the curve depicting glutaminase activity when 6 rats were begun on $NaHCO_3$. The observed values obtained 24 hours later are enclosed at the tip of the arrow.

The rise in renal glutaminase activity in the rats drinking 1.6 per cent NH_4Cl is demonstrated in Figure 3. The effect of allowing the rats free access to $NaHCO_3$ for 24 hours is illustrated in Figure 4. The results obtained seem related to renal glutaminase activity.

Summary

Renal glutaminase activity in rats was stimulated by administration of the acidifying salt, ammonium chloride. Addition of this substance to the drinking water brought about a condition of susceptibility to coliform infection of the kidney. There was no evidence of a generalized effect on resistance. There appeared to be a correlation between the degree of susceptibility and the level of glutaminase activity. The findings are consistent with the thesis that ammonia formation in the kidney inactivates complement and thus renders the organ more susceptible to certain kinds of infection.

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GENERAL DISCUSSION

Dr. Braude: *I should like to mention some clinical observations that might support the experimental observations described by Dr. Rowley and Dr. Beeson.*

First, in patients whom we have studied with chronic pyelonephritis, we have observed a reduction *in the bactericidal power of serum against* the organism isolated from their own urine, and one of the interesting things is that in this group there are patients with cirrhosis of the liver, who might be expected to have an elevation in blood ammonia and therefore have the basic circumstance that would fit clinically with the experimental observations that Dr. Beeson described.

Earlier we had observed a number of cases of disseminated *Hemophilus influenzae* infection in adults, a rather unusual thing because adults are usually considered to be much more resistant to this infection than are children It turned out that the three people whom we studied extensively with this condition were alcoholics who had severe liver disease, and who might be expected to have elevated blood ammonia levels, although we did not measure them. In addition, in each instance, the bactericidal power of the serum of these people was reduced.

The second observation I wish to describe, which supports Dr. Rowley's idea of the importance of phagocytosis in resistance against urinary infection, concerns the condition of the leukocytes in people with pyelonephritis *It is surprising, really, since there is so much pyuria in* this disease, that these cells are not able to contribute more effectively to recovery, yet we see patients who have persistent infections despite a good leukocyte response, and in whom the disease even progresses.

So we did a simple experiment. We stained these cells with a supravital stain, neutral red, which is known to stain the nuclei only of dead cells, and to our surprise we found that practically all of the cells in the urinary sediment in pyelonephritis took the stain and were dead or nearly so.

Dr Rantz I should like to comment briefly on bactericidal effects in relationship to disease.

Dr. Robert Roantree, when he was working in our laboratory, showed [illegible] blood in cases of [illegible] effect of sera. [illegible] pens there were a considerable number of Escherichia represented.

We were somewhat disappointed and disturbed to discover that organisms isolated from the urine had very much the same distribution

tion and tissue concentration which account for the increased susceptibility.

Just as added proof, I wonder if one might be able to distinguish the effect of ammonia from acidification (and the subsequent change in glutaminase activity) by administering to rats precursor amino acids which would increase ammonia production by virtue of increasing substrate concentration, without the simultaneous change in glutaminase activity. I think in this way one could more easily distinguish between these possibilities

I have one other comment Knowing little about the problem of pyelonephritis until I arrived here, I have learned that the medulla is more susceptible to infection in the experimental animal than is the cortex

In so far as your thesis is concerned, Dr. Freedman, I think it is interesting that the concentration of glutaminase is considerably higher in rat cortex than it is in the medulla and the papilla, again arguing against glutaminase activity per se as the pertinent factor and in favor of the changes in ammonia concentration.

DR. FREEDMAN I should like to say first that I am told by Dr Rowley that the ammonium ion has no effect on the fourth component of complement, that this is only an effect of ammonia. Despite this, the ammonium acetate experiments were carried out to control the ammonium that was administered as NH_4Cl, with the realization that ammonium acetate would do no more than give ammonium ion, which would then be converted to urea and have nothing to do with ammonia production in the kidney

With regard to potassium deficiency and Dr. Relman's question, there is some dispute about the susceptibility to infection in potassium deficiency Also this experimental model is complicated by the fact that in potassium deficiency one gets visible morphologic lesions in the medulla of the kidney which eventually will produce susceptibility to infection, this is not a clear-cut situation.

The point I want to emphasize, however, is that I am sure many things that will increase renal glutaminase activity will not produce susceptibility to infection We are going here from an experimental model, in which the organism does nothing, to one in which it can produce infection To accomplish this, the renal ammonia-forming mechanism has to be stimulated enormously.

With regard to Dr Orloff's question, four days of ammonium chloride do not produce much change in susceptibility, it usually takes a lot more than that. Body pH might not play much of a role in this increased susceptibility since the pH adaptation to ammonium chloride would be

expected to increase with time—that is, the pH change in the body would be less as time went on, whereas the ammonia production would increase. This would seem to indicate, therefore, that ammonia production would be the more significant of the two phenomena.

In addition, the pH change is a systemic one, yet evidence of infection is found only in the kidney and not in other organs

Dr Rector To elaborate further on a point raised by Dr. Orloff, I should like to emphasize that not only is the concentration of glutaminase considerably higher in rat cortex than in medullary papilla, but also that during ammonium chloride acidosis the adaptive increase in glutaminase activity occurs only in the cortex and does not involve the medulla However, the ammonia content of the medullary tissue goes up quite markedly in ammonium chloride acidosis. This suggests that the concentration of ammonia rather than the activity of glutaminase is responsible for Dr Freedman's observations

Dr Beeson Dr Rowley had to leave to return to England He wanted me to tell Dr Schreiner that he does not know the answer to his question, and I am sure I don't either.

I thank Dr. Rantz for having already done the studies on the serum sensitivity of coliform from urinary tract infections, because we were about to undertake a long, tedious study of this kind

The business of the high ammonia level in cirrhotics has occurred to us, but someone will have to find out whether or not there is any real lowering of the blood complement in patients with high blood ammonia levels, and I am not sure that will be true

In regard to Dr Orloff's point, I realize that there is argument about this, and that glutaminase can certainly be demonstrated throughout the nephron, in fact, we found no difference in anticomplementary activity of cortex and medulla when we tested them by that method.

I suppose, like all scientists, we are inclined to accept the evidence that favors our thesis In this case the evidence we are inclined to accept is that of Richterich and Goldstein, and of Walker and Pitts and coworkers, that although glutaminase is present throughout the nephron, the only place that ammonia seems to be added is low in the nephron, and this seems to us to be the key point here.

We would not deny for a moment that there is glutaminase higher in the nephron, or that it is present in a lot of other tissues, but it is quite possible that glutaminase is doing something else in protein metabolism than making ammonia, because the only place one can demonstrate ammonia formation is low in the nephron

the ureter might do the same thing. At the present moment, however, there is danger that the rest of the profession may lose sight of this fundamental and very rational concept, and ignore it in their enthusiasm for the marvelous and still comparatively new antibiotic drugs. The onus of preserving a rational balance in what we might call the popular attitudes toward these complex matters falls on the urologists

Stasis of urine may be due not only to obstruction but also to lack of expulsive power from affections of the nerves supplying the muscular coats of the bladder or ureters. Everyone is familiar with the utterly intractable character of infections occurring in such cases, unless and *until the* emptying power is restored. This kind of stasis may well be called adynamic stasis. It may or may not be amenable to treatment, but its most important aspect is that it is sometimes very difficult to diagnose. Aside from the various examinations which can be made to determine the state of the central and peripheral nervous systems, I can only remind you of the cystometric test.[9] The information it gives is exact and reliable, and its application is simple.

Obstructions *at the neck of the bladder often cause frequent* and difficult urination, but in mild cases these symptoms may be slight or absent, yet the obstruction be sufficient to keep up active infection in the bladder or prostate or both

Of the symptoms usually ascribed to ureteral obstruction, pain, either constant or colicky, is the commonest, but when it is absent there may be little to suggest that the ureter is obstructed. Such painless obstructions may be severe enough to cause extensive hydronephrosis; even if less severe they may serve to keep up chronic urinary tract infections, they may be the most important factor in the production of stones, or they may possibly cause other diseases of the kidney now regarded as of *unknown origin.*

The relationship between obstruction and infection is very clear and I am surprised that anyone would any longer argue seriously against it I cannot tell you who first drew attention to this relationship, but it was certainly foreshadowed by many urologists who made the *contributions* upon which the present knowledge of the relationship is based. Hunner[7] showed very early how renal infections cleared as a result of ureteral dilatation rather than of pelvic lavage. William R. Stevens[11] devised an instrument for calibrating the female urethra, and noted that *if the* size was smaller than 28 French, persistent infections were apt to occur. Hinman in his *Principles and Practice of Urology*[6] expounds the relationship excellently and at length. The relationship can be expressed by two statements self-evident to experienced urologists.

They are: first, "obstruction predisposes to infection." That is to say, any part of the urinary tract lying above an obstruction, and therefore

subjected to the pathologic and anatomic effects of obstruction, becomes infected more easily than the normal urinary tract, however the infectious agent may reach the tract. Second, "obstruction perpetuates infection." That is to say, obstruction destroys the natural ability of the normal, unobstructed urinary tract to throw off infection, so that infections above obstructions become chronic, and indeed permanent. By the same token, infection above obstruction is resistant to treatment and can seldom be permanently cured, no matter what form of treatment is used, unless and until the obstruction is removed.

The normal bladder and urethra are so constructed that, in the adult, the bladder will hold up to 450 or 500 cc. when filled to capacity, and that when voiding occurs the bladder will be completely emptied before the detrusor muscle becomes fatigued.[4] Cystometric studies show that a normal bladder can produce an internal pressure not exceeding a maximum of 50 or 60 mm Hg. The urethra therefore must be of such size that at this pressure the entire bladder contents can flow through it in about 20 seconds – that is, at the rate of at least 22 to 25 cc. per second. The validity of this hypothesis can be tested with an instrument called the uroflowmeter, designed and constructed and described by Dr. Willard M. Drake, Jr.,[6] about ten years ago. Study of a large series of presumably normal individuals showed that the mean rate of flow was between 22 and 28 cc. per second, perhaps a little higher in females than in males. A few had rates as high as 50 cc. per second, while those with rates lower than 22 cc. per second had definite evidence of obstruction.

When the urethra becomes obstructed at any point or for any reason, the rate of flow is diminished, and the only way it can be restored to normal is by a stronger detrusor contraction, producing a voiding pressure in the bladder higher than normal – in fact high enough to force the urine through the constricted urethra at a higher velocity, so that it flows at a normal rate, that is, a normal number of cubic centimeters per second. If the neuromuscular apparatus is normal this reaction on the part of the detrusor actually occurs, and if it continues long enough the detrusor becomes hypertrophied, so that the bladder wall is thickened and the voiding pressure markedly elevated, even to 100 mm Hg or higher. As long as this increased pressure enables the urine to flow through the urethra fast enough to empty the bladder before the detrusor becomes fatigued, there will be no residual urine and we may say the bladder is compensated, just like a hypertrophied heart. These reactions all occur automatically, and the patient is entirely unaware of the early states of obstruction.

Partial and even slight obstructions in the upper tract also make organs above them susceptible to infection and tend to perpetuate it after its onset.

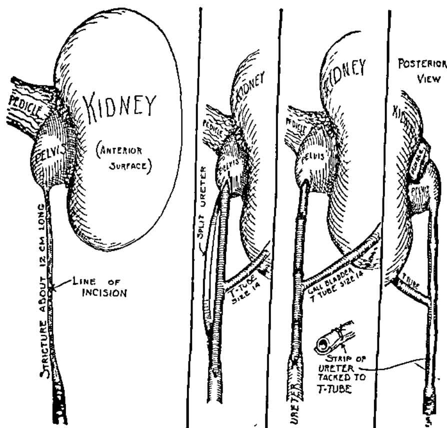


Figure 6 Case 2 Operative findings The two middle panels show method of intubating ureter with T-tube, right panel shows posterior view. Ureteral splint remained in place 4 weeks

The second patient (Case 2) was ill with an extensive right calculous pyonephrosis The left kidney was investigated by ureteral catheterization and retrograde pyelography. The urine obtained was clear and free of leukocytes and bacteria, but immediately following this catheterization the patient experienced severe pain in the left kidney, and chills and high fever began. The pyelogram (Figure 5) showed a moderate left hydronephrosis and a very narrow upper ureter. Two weeks of the most intensive medical treatment brought about no improvement, so that operation was undertaken. The ureter was found to be extremely small for a distance of 12 cm. (Figure 6) and was treated by intubated ureterotomy. Convalescence was uninterrupted. The right [illegible] bly damaged, was removed shortly [illegible] An intra[illegible] (Figure 7) made 7 years later [illegible] ently [illegible] ney and ureter.

It is now 19 years since operation. The patient has enjoyed perfect health, and the urine, examined yearly, is always clear and free of leukocytes and bacteria. Renal function tests are normal.

The third patient (Case 3) at age 49 had right-sided pain and fever leading to the removal of a normal appendix. Investigation then showed a right hydronephrosis due to a ureteropelvic obstruction, heavily infected and



FIGURE 7. Case 2. Intravenous urogram, 5-minute film, [illegible] years after operation. Right kidney has been removed. Note normal outlines of pelvis and ureter. Urine sterile, renal function normal, patient perfectly well.

containing a calculus (Figure 8). Two eminent urologists advised nephrectomy. At operation the stone was removed and the ureteropelvic junction enlarged by intubated ureterotomy. The right panel (of Figure 8) is an intravenous urogram taken 6 months after operation. It is interesting that a few bacilli could be found in the urine for 4 months

after operation, but there were no symptoms and no medication was given. Since then, now 12 years ago, the patient has been perfectly well and the urine free from leukocytes and bacteria.

Such cases can be cited almost endlessly. Figure 9 (Case 4) shows a similar condition in a male patient, now well, with clear urine and without treatment for 8 years. In another case (Figure 10, Case 5) there were two

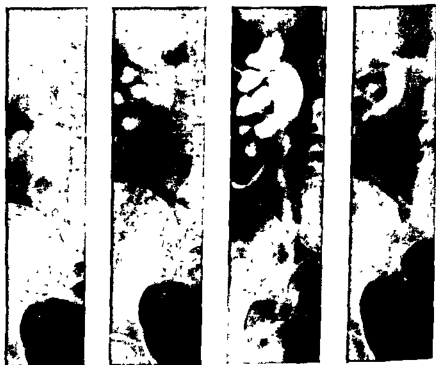


FIGURE 8 Case 3 First panel renal calculus Second panel intravenous urogram showing right hydronephrosis, before operation. Third panel antegrade right pyelogram made at time of withdrawing splint tube, 4 weeks after operation Fourth panel intravenous urogram of right kidney, 6 months after operation. Urine sterile, patient perfectly well.

large stones in a supernumerary left ureter (one of them in a ureterocele). The ureter above the stones was hugely enlarged and thickened, with its lumen full of thick pus. After removal of the two stones and of the ureterocele, the affected ureter returned to normal (as shown in Figure 11). At this time, 8 months after operation, all three ureters were catheterized and clear, sterile urine was obtained from each. The patient has been perfectly well for 15 years since operation.




FIGURE 9 Case 4 Left panel intravenous urogram showing right hydronephrosis, before operation Right panel intravenous urogram showing right kidney 8 months after operation Urine sterile, patient perfectly well




FIGURE 10 Case 5 Left panel film showing large stone in left ureter, smaller stone in left ureterocele Right panel bilateral retrograde pyelogram, before operation. Ureter to upper left pelvis could not be catheterized.

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[illegible]

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When lower urinary tract obstruction is severe and long-standing, the increased bladder pressure may interfere sufficiently with the expulsion of urine from the ureters into the bladder to bring about hydronephrosis and hydro-ureter (Figure 12, Case 6). Removal of the obstruction at the vesical orifice is all that is necessary to bring about return to normal, both anatomic and functional, of the renal pelvis and the ureter, as shown in the right panel of Figure 12. Along with this the urinary tract infection disappears and does not return, provided the removal of the obstruction is complete. Similar situations can arise in women. A vesical orifice obstruction can give rise to a pulsion diverticulum before there is any roentgenologically demonstrable change in the upper tract. In such cases,

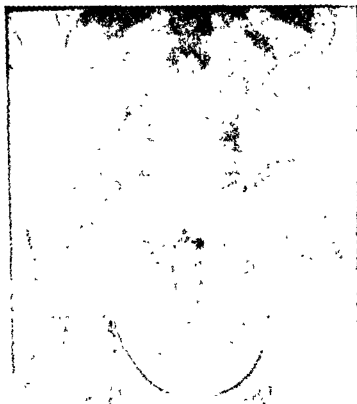


FIGURE 13. Case 8. Intravenous urogram showing heavily trabeculated bladder, right hydronephrosis and hydro-ureter, reflux filling of left ureter. Left kidney has been removed.





FIGURE 11. Case 5. Intravenous urogram 8 months after operation Note that upper left pelvis is now functioning well, outline same as on right side. Urine from both left ureters clear and sterile, patient perfectly well





N--- bilateral
teral has
. isurethral
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obstruction A most thorough study, with cystoscopy, cystometry, calibration of ureters and urethra, uroflowmetry, and intravenous and retrograde pyeloureterography, as well as cystography, with proper post-evacuation films, is necessary. Until very recently, this would have been

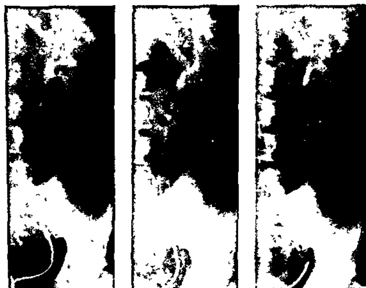


FIGURE 17 Case 11 Painless hematuria from left kidney Left panel left retrograde pyelogram, thought at the time to be normal Middle panel similar pyelogram made 6 months after dilatation of ureter to size 14 French Right panel similar pyelogram made 12 months after dilatation All pyelograms made by gravity method with the same pressure It is now evident that the left panel shows a hydronephrosis

the best we could do, and it would have disclosed a large number of slight and obscure obstructions Now, however, we can do very much better, thanks to the work of an internist, Fredrik Kiil* of Oslo, Norway – truly a "bahnbrechende Arbeit" For the first time we begin to have a picture of how urine is really conducted from the renal pyramid to the exterior, and an idea of what the intrapelvic pressure really is, both normal and pathologic Moreover, the methods devised and utilized by Kiil are simple and well adapted to everyday clinical work in any well-equipped urology department.

The results to date are clear-cut and, to many of us, startling in their unexpectedness The normal ureter is a pump of amazing power and efficiency, developing pressures of from 20 up as high as 80 mm Hg in [illegible]

without benefit of antibiotics, as this was before the advent even of sulfanilamide.

Two other cases represent a commonly mismanaged similar condition in males

The first, a man of 72, underwent right nephrectomy, apparently for infected hydronephrosis, 23 years ago. Eight years ago recurrent attacks of infection with burning and frequency began Treatment was entirely medical. *Cystoscopy showed prostatic obstruction, but operation was not* recommended, I suppose on the ground that the prostate was not enlarged to rectal palpation, and that there was only one kidney. Finally I performed transurethral resection. Within 3 weeks the urine was clear and has remained so since.

The second, a man of 44, had had definite frequency all his life, to which he became so accustomed that he thought he was normal. Five years before I saw him, definite infection began with pyuria. Persistent treatment over the entire 5 years with prostatic massage, irrigations, urethral sounds, and all available antibiotics, as well as vasotomy with injections, produced no improvement The patient was voiding from 8 to 18 times each night, and every hour in the daytime. The diagnosis of congenital contracture of the vesical orifice was obvious Transurethral enlargement of the orifice was carried out 4 years ago. Within 10 or 12 weeks the urine was clear and sterile, the patient was voiding only 3 or 4 times in the day and not at all at night, and he had gained 14 pounds. This excellent condition continues today.

There can be no doubt that the failure of treatment in many cases of urinary tract infection, or the so-called chronic pyelonephritis, is due to the failure to recognize slight and obscure obstructions. The last-cited case is an excellent example of unrecognized lower tract obstruction, and Figure 17 (Case 11) shows a striking example of unrecognized ureteral obstruction.

The patient, a physician, experienced hematuria from the left kidney. The retrograde pyelogram in the left panel was interpreted as normal In the absence of any positive finding except some resistance to the passage of a #7 catheter, the ureter was dilated on several occasions, reaching size #14 The other panels represent pyelograms made 6 and 12 months later All were made by the gravity method with identical pressures and are therefore exactly comparable. It is now quite clear that the first picture represented a hydronephrosis. This occurred 20 years ago, and the patient has remained in perfect health ever since without any treatment.

In a letter to me Edward Kass says "The present methods for detecting obstruction seem to have reached an end point." I wish to deny this allegation categorically and most emphatically. A single intravenous urogram, interpreted by a roentgenologist, is certainly not sufficient to rule out

Hospital, operated upon her in 1916, closing the vaginal opening as well as the urethra, thus creating for her a cloaca. This woman, now 79 years of age, has a direct communication between bladder, vagina, and rectum which is well demonstrated in the accompanying roentgenogram (Figure 1) made after injecting contrast medium with a rectal tube. The pyelograms (Figure 2) made in 1938 – 22 years after her operation – demonstrated no abnormality. She returned to our hospital in 1956 for an operation to remove an esophageal diverticulum and on that occasion we



FIGURE 1 A 79-year-old woman with direct communication between the bladder, vagina, and rectum. In 1916, vagina and urethra were closed, creating a cloaca. Above x-ray taken in 1958 after injection of contrast medium by rectal tube

whom the urine was found to remain free from bacteria as a result of continuous acid irrigation of the bladder. This and the use of continuous irrigation with other antiseptic agents will no doubt lessen significantly the bacterial flora of the bladder that is on indwelling catheter drainage, but the total avoidance of bacterial invasion even under the best of conditions cannot be expected. The clinician who aspires to the avoidance of bacterial invasion in these circumstances must remember an ancient admonition of our guild: "That the catheter drains the bladder but not the urethra."

The surgeon who performs operations for the treatment of prostatism is aware that the avoidance of infection in the urine during the immediate postoperative period can never be attained, and that the employment of antibacterial agents will not effect this end. On our own service many such efforts have demonstrated the futility of achieving a sterile urine during the immediate postoperative period, yet acute pyelonephritis is an uncommon complication of prostatectomy.

Talbot[15] reported his observations on 59 paraplegic patients who were on catheter drainage for periods of from 1 to 14 years. All had bacilluria but 72 per cent had no clinical evidence of renal disease. He believes[14] that the occurrence of significant renal infection is not so much due to the indwelling catheter as to the development of obstructive lesions in the upper urinary tract.

We have followed the course of large numbers of patients who have been placed on catheter drainage following abdominal perineal operations for cancer of the rectum and for gynecologic operations, and have observed that these patients invariably have infected urine during and following catheter drainage, yet pyelonephritis rarely complicates these cases, and persisting urinary infections in these patients will usually be found to be due to obstructive disease or inflammatory lesions of the urethra.

Two cases in my experience will give further emphasis to the perplexing nature of the etiology of so-called "ascending pyelonephritis"

One was that of a medical student who had been followed in our clinic during his premedical years for recurrent episodes of bladder infection. Cystoscopy had been performed on several occasions and ureteral catheter specimens of urine from both kidneys were always normal. Eventually we found that the cause of his cystitis was a communication between the vermiform appendix and the bladder, and he was cured by appendectomy. This man had fecal contamination of his bladder urine for several years, yet he never developed pyelonephritis.

The other patient is a woman who had a childbirth injury that produced a tear involving the bladder, the vagina, and the rectum. The late Professor Reuben Peterson, Chief of Obstetrics in the University of Michigan

a kidney which is the site of hematogenous coccal infection becoming secondarily invaded by bacillary organisms. This has been experimentally demonstrated by de Navasquez

The patient with a continuing bacilluria lives in constant danger of renal involvement. Yet observations on hundreds of such patients reveal that, although many develop clinical evidence of kidney infection, this is not inevitable It may be that those who seem to escape — and they are now the majority — harbor latent pyelonephritis The finding of normal kidneys at postmortem in some of these patients, however, indicates that this is not necessarily the case.

I must point out that the clinical material I have observed is made up entirely of what Dr Brod this morning called secondary cases. It is, perhaps, justifiable to speculate upon whether any of the primary cases have had episodes of urinary disease which are lost in the past I can think of one in which the only evidence at postmortem was fibrosis of the ureter revealed by differential stain Nonetheless, the disease in young females that Dr Freedman reported on this morning may represent a distinct entity from the type of kidney infection I most often see Yet I am willing that both should be called pyelonephritis A further study of those in whom renal infection is obviously present has yielded certain clues as to the circumstances in which it develops. It has been my opinion that in most of these cases the route of invasion was along the wall of the ureter, particularly through the subepithelial tissues which are continuous from the bladder into the ureter and eventually into the interstitium of the kidney. The observations leading to this conclusion have already been published and need not be presented again at this time[1] On the basis of earlier reports, I had not been impressed by the likelihood of significant invasion along the periureteral lymphatics, but the work of Dr Murphy, shown yesterday, goes far toward converting me to accepting this as a possible route. It is of particular interest that the development of abnormally high intravesical pressures, which seems to be such a potent factor in ureteral invasion and dysfunction, is also a determinant in favor of ascent of particular matter

Dr Davis has clearly indicated the importance of functional obstruction, which may not be easily recognized, and emphasized the importance of studying the dynamics of the urinary tract This has, in fact, been a major preoccupation of mine for some years Such studies must include the ureter as well as the bladder, and ordinary methods of calibration may be of no value because there is no narrowing of the lumen. To the methods enumerated by Dr. Davis, all of which are valuable, I should like to add one which we have found particularly revealing, namely, fluoroscopy with its recent inclusion of cinefluorography The ureter as well as the bladder may decompensate. More particularly, its dysfunction may in-

DESIGNATED DISCUSSION

Herbert S. Talbot, M.D.
(West Roxbury, Massachusetts)

Dr. Davis' and Dr. Nesbit's papers are important contributions to a better understanding of certain factors in the development of pyelonephritis. Consideration of the relation of instrumentation to renal infection and of the part played by obstruction in the etiology of pyelonephritis leads us squarely to two fundamental questions. First, how do the organisms reach the kidney? Second, what factors render the kidney susceptible to invasion?

Before I go further, let me state that I do not intend to devote this discussion to a defense of the catheter. I am not sure that it needs any defense, because the correct use of the catheter has not been under attack. It is not my understanding that the careful observers who have, in recent years, called attention to hazards associated with its unnecessary or incorrect use had any intention of proscribing it altogether. Unfortunately, there is a tendency to assume that the best way to correct the abuse of any agent or activity, however valuable, is to do away with it. When one finds a house officer hesitant to catheterize an old gentleman whose bladder is approaching the level of his umbilicus, one is entitled to conclude that, as has happened so often before, sound gospel is being misinterpreted.

It cannot be denied that repeated or continued urethral catheterization leads almost inevitably to infection of the bladder urine. As Dr. Nesbit has pointed out, the onset of such contamination may be delayed and its incidence perhaps in some degree reduced by certain measures, but in the present state of our knowledge and skill, the association cannot be gainsaid. On the other hand, it is also true that bacilluria is frequently found in patients who have never been catheterized or instrumented. In some instances no etiologic factor is apparent, but often there is an element of obstructive uropathy or some alteration in the ability of the tissues to resist bacterial invasion, as seems to occur in diabetes mellitus.

Turning now to the first of the two questions I have proposed – How does the infecting organism reach the kidney? – the accumulation of clinical and experimental evidence suggests that if it is a coccus it may, and probably most often does, travel via the blood stream. In the case of the bacillary organisms which are most frequently associated with clinical pyelonephritis, however, the preponderance of evidence is that the kidney successfully resists hematogenous invasion unless it has been previously damaged, or there is urinary obstruction, or the kidney involvement is one aspect of an overwhelming bacteremia. There may be, as Dr. Nesbit has so aptly pointed out, a combination of these two types of infection,

DR. YOW. Needle aspiration of the bladder can be performed with no significant morbidity. It is essential that the patient's bladder be sufficiently full to extend above the symphysis pubis. This can best be accomplished by carrying out the procedure before the patient voids in the morning, but it may be done at any time during the day by forcing fluids until the patient is definitely aware of his bladder being distended. The greatest difficulty encountered in the procedure is with the female patient who has a cystocele. Even a moderately severe cystocele may allow the bladder to contain 500 or 600 cc. of urine without extending above the symphysis. Under these circumstances it is necessary to have someone manually lift the bladder through the vagina while the needle is being inserted into the bladder superpubically.

The exact technique used in my laboratory has been as follows: After the superpubic area is cleansed with an antiseptic solution the area immediately above the symphysis and in the midline is infiltrated with a 1 per cent Novocain solution, and a 22 gauge spinal needle is inserted posteriorly and inferiorly. When the bladder is punctured the urine can be aspirated without difficulty. There have been no complications from the procedure performed on approximately 100 patients, though in the beginning of the study the bladder was missed on several occasions when cystoceles were present. Even under these circumstances, however, no difficulties arose from needling the area. Several male patients stated that they preferred the procedure to catheterization, but on the other hand the need to utilize the procedure for diagnostic purposes in males is less frequent because of the fact that a clean voided specimen is usually satisfactory bacteriologically.

While our primary reason for collecting urine by bladder aspiration was to determine to what extent urethral contamination influenced bacterial counts of the urine, it is possible that there may be other indications for this procedure. It may be highly desirable in an occasional patient to determine the significance of a low bacterial count of the urine. Pyelonephritis due to organisms such as the enterococcus and staphylococcus certainly may be associated with low colony counts. A voided specimen or a specimen collected by catheterization cannot be considered reliable for investigating this possibility, since both may be associated with contamination with these organisms. The possibility of low colony counts arising from chronic infections of the periurethral glands may also be studied more carefully by comparing voided and aspirated urines. Under some circumstances it may be safer to aspirate the bladder urine than risk the possibility of infecting the patient with a catheter. This is most likely to be of significance in patients who have an unusual susceptibility to pyelonephritis, such as patients with severe diabetes.

women for culture in our hospital. In no instance has the introduction of the catheter for this purpose been followed by urinary tract infection when it has been done by the careful techniques used by these people

The reason it was necessary to train people for this purpose, to insure that by careful sterilization of the perimeatal area we would not introduce organisms, was that a number of patients in our hospital were too sick to cooperate with the person who collected the urine by the methods that have been advocated for culture without use of the catheter.

In order to standardize this procedure in a big hospital, where as many as sixteen to thirty urines were collected per day for culture, it was necessary to use the catheter, and I can report to you that we have been extremely successful in introducing the catheter for collecting urines without establishing infection.

DR KEITEL I am sure that all of us appreciate the importance of finding obstruction as a primary initiating etiology in pyelonephritis I should like to know, however, what percentage of patients referred to urologic surgeons with pyelonephritis are found, in their hands, and studied with methods that are presently available, to have obstructive lesions

DR NESBIT I can't answer the question.

DR DAVIS· Probably I would be regarded as an extremist in this matter, but it has seemed to me through many years of practice that obstructions are so common in persistent and persistently recurring infections that I would serve the interests of my patients better to assume that whenever there was a persistent or recurrent infection there was an obstruction—which would force me to continue searching for it until I found it

I could not give you an exact figure, but the percentage, I might say, is very high, and that includes also the percentage of successful treatment of chronic and recurrent infections

DR. MAXWELL I think Dr Talbot made an excellent point when he said that nobody is against the use of the catheter when it is indicated Like most other people, we try not to use it when it is indicated, but occasionally we have to.

I was interested to notice that Dr. Yow is the only speaker thus far who has talked about puncturing the bladder. Our colleagues in cardiology often stick needles through the chest wall to measure the pressure in the left ventricle. I should like to ask Dr. Yow to give us more details about direct bladder puncture, and whether he had any morbidity, and the potentialities of this instead of catheterization when indicated

DR. YOW: Needle aspiration of the bladder can be performed with no significant morbidity. It is essential that the patient's bladder be sufficiently full to extend above the symphysis pubis. This can best be accomplished by carrying out the procedure before the patient voids in the morning, but it may be done at any time during the day by forcing fluids until the patient is definitely aware of his bladder being distended. The greatest difficulty encountered in the procedure is with the female patient who has a cystocele. Even a moderately severe cystocele may allow the bladder to contain 500 or 600 cc of urine without extending above the symphysis. Under these circumstances it is necessary to have someone manually lift the bladder through the vagina while the needle is being inserted into the bladder superpubically.

The exact technique used in my laboratory has been as follows. After the superpubic area is cleansed with an antiseptic solution the area immediately above the symphysis and in the midline is infiltrated with a 1 per cent Novocain solution, and a 22 gauge spinal needle is inserted posteriorly and inferiorly. When the bladder is punctured the urine can be aspirated without difficulty. There have been no complications from the procedure performed on approximately 100 patients, though in the beginning of the study the bladder was missed on several occasions when cystoceles were present. Even under these circumstances, however, no difficulties arose from needling the area. Several male patients stated that they preferred the procedure to catheterization, but on the other hand the need to utilize the procedure for diagnostic purposes in males is less frequent because of the fact that a clean voided specimen is usually satisfactory bacteriologically.

While our primary reason for collecting urine by bladder aspiration was to determine to what extent urethral contamination influenced bacterial counts of the urine, it is possible that there may be other indications for this procedure. It may be highly desirable in an occasional patient to determine the significance of a low bacterial count of the urine. Pyelonephritis due to organisms such as the enterococcus and staphylococcus certainly may be associated with low colony counts. A voided specimen or a specimen collected by catheterization cannot be considered reliable for investigating this possibility, since both may be associated with contamination with these organisms. The possibility of low colony counts arising from chronic infections of the periurethral glands may also be studied more carefully by comparing voided and aspirated urines. Under some circumstances it may be safer to aspirate the bladder urine than risk the possibility of infecting the patient with a catheter. This is most likely to be of significance in patients who have an unusual susceptibility to pyelonephritis, such as patients with severe diabetes.

women for culture in our hospital. In no instance has the introduction of the catheter for this purpose been followed by urinary tract infection when it has been done by the careful techniques used by these people.

The reason it was necessary to train people for this purpose, to insure that by careful sterilization of the perimeatal area we would not introduce organisms, was that a number of patients in our hospital were too sick to cooperate with the person who collected the urine by the methods that have been advocated for culture without use of the catheter.

In order to standardize this procedure in a big hospital, where as many as sixteen to thirty urines were collected per day for culture, it was necessary to use the catheter; and I can report to you that we have been extremely successful in introducing the catheter for collecting urines without establishing infection.

Dr. Keitel I am sure that all of us appreciate the importance of finding obstruction as a primary initiating etiology in pyelonephritis. I should like to know, however, what percentage of patients referred to urologic surgeons with pyelonephritis are found, in their hands, and studied with methods that are presently available, to have obstructive lesions.

Dr Nesbit I can't answer the question.

Dr Davis Probably I would be regarded as an extremist in this matter, but it has seemed to me through many years of practice that obstructions are so common in persistent and persistently recurring infections that I would serve the interests of my patients better to assume that whenever there was a persistent or recurrent infection there was an obstruction — which would force me to continue searching for it until I found it.

I could not give you an exact figure, but the percentage, I might say, is very high, and that includes also the percentage of successful treatment of chronic and recurrent infections.

Dr Maxwell: I think Dr Talbot made an excellent point when he said that nobody is against the use of the catheter when it is indicated Like most other people, we try not to use it when it is indicated, but occasionally we have to.

I was interested to notice that Dr. Yow is the only speaker thus far who has talked about puncturing the bladder. Our colleagues in cardiology often stick needles through the chest wall to measure the pressure in the left ventricle. I should like to ask Dr. Yow to give us more details about direct bladder puncture, and whether he had any morbidity, and the potentialities of this instead of catheterization when indicated

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DR. SCHREINER: I think this conference has illustrated one of the beauties of bringing together people from different backgrounds. If one reads some of the infectious disease literature, as our students do, one comes away with the idea that pyelonephritis is a disease of the urine instead of a disease of the kidney.

If, on the other hand, one listens to some of our surgical colleagues who are ardently in the defense of their pointed weapons, one gets the idea that unless a patient has end-stage uremia, or a temperature of 106° F., he has no postinstrumentation pyelonephritis.

It is for this reason that we have chosen to describe this disease recently as a clinical and histologic spectrum, and I think what we are seeing today are various grids taken out of this continuous spectrum.

One can certainly see patients of the asymptomatic variety that Dr Kass has described. We have biopsied people (as has Dr. Yow) for other reasons than suspected infection. One notable reason was our study of preeclamptic patients, in whom we found collections of leukocytes in the renal cortical areas, suggesting that this can happen in the absence of bacteria in the urine These people did not have positive smears or high colony counts

On the other hand, I think Dr. Kass has provided an excellent diagnostic approach, and we should maintain some humility about this disease—*which seems to me to be best considered as a continuous spectrum*—and that we should emphasize all of the preventive aspects.

I certainly enjoyed Dr. Talbot's comments on functional integrity of the lower urinary tract. We have an institution called the Soldiers' Home in Washington, in which there are elderly gentlemen who have been walking around the lawn for years with indwelling catheters, who do not have debilitating disease of the upper urinary tract; and one of the reasons, I think, is that these patients maintain functional integrity.

On the other hand, we have seen patients (as have all of you) develop severe infection while lying on a hospital bed and having an indwelling catheter put in for only a matter of hours. Here again, we have to consider functional integrity

My question to Dr. Nesbit and Dr. Talbot is prompted by the fact that in the past two years we have studied some thirty-five cases of young children who had no obvious anatomic difficulties of the bladder neck, and many of whom would not be accepted by urologists as having obstructive disease of the lower urinary tract, but who had minimal bladder trabeculations. Internists are used to thinking of left ventricular hypertrophy as being due to work against pressure. I should like to ask how highly they would regard trabeculation in children as a sign of functional hypertrophy.

DR. TALBOT I think the presence of trabeculation is at least strong evidence in favor of the fact that there is obstruction at the bladder neck. I think there are very few urologists who would not agree with that. Whether it is in itself a sufficient indication for surgical relief would depend on other findings in the individual case, and these are the children who should be studied by cystometry and cystogram and possibly by fluoroscopy.

I might add that one of the most profitable methods of study is a very careful investigation of the functional pattern of micturition that any individual child or adult normally follows This can yield very useful information. It is perhaps unspectacular It is easy to get, and it does not involve any pointed weapons

SUMMATION

GEORGE GEE JACKSON
(Chicago, Illinois)

I am not going to attempt to summarize the afternoon's papers and the discussion of them There are some points, however, I should like to emphasize.

We anticipated yesterday the fact that clinical bacteriology in urinary tract infections was not done with the precision that we as scientists expect and demand in other areas of investigation, Dr Edwards has given us again a good look at the precision we should use in the identification by bacteriology There are many among us who would not think of making a diagnosis on a patient without looking at his urine, but few among us who are able by training or interest to do the bacteriology in equally critical fashion.

The fact that the literature is quite heavily loaded with the mistakes of the type to which Dr Edwards referred is, I think, clear to anyone who reads far in this literature There is, for example, from an outstanding clinic the report that something like 50 or 60 per cent of *Escherichia coli* associated with genitourinary infections hydrolyze urea These almost certainly are mixed infections, with *Proteus* species Sometimes we have had to pick single colonies of *E coli* through five generations before we could identify the contaminating *Proteus*. This is not an easy job, and it isn't just the amateur who is easily misled, but we do need to pay attention to increased precision in our bacteriologic methods

Such attention might be productive of a better understanding of pyelonephritis. Yesterday it was suggested that urease may have some role in the infectivity of strains We have found that among patients with initial clinical infections, only 30 or 40 per cent of the strains recovered during these infections ferment salicin or sucrose, whereas 90 to 100

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*Bacteriuria, Pyelonephritis, and Hypertension: A Clinical and Pathologic Study**

HANS G. GRIEBLE, M.D., and GEORGE GEE JACKSON, M.D.
(Chicago, Illinois)

Bilateral contracted pyelonephritic kidneys were recognized as a cause of Bright's disease as early as 1882.[44] Lohlein[30] later described this variety of contracted kidneys as the pathologic end-stage of chronic urinary tract infection. In that work, the clinical hallmarks of pyelonephritis such as pyuria, bacilluria, mild proteinuria, a paucity of casts and red blood cells in the urinary sediment as well as its association with arterial hypertension with cardiac hypertrophy, were clearly outlined. These clinical and pathologic findings subsequently have been amply confirmed in numerous autopsy series including the striking association of pyelonephritis and hypertension.

The etiologic role of pyelonephritis in the causation of hypertensive disease was accepted by most students of the disease.[1, 5, 6, 7, 11, 14, 20, 30, 32, 34, 37, 41, 42, 45, 49, 50, 51] On the other hand, essential hypertension with ensuing vascular changes in the kidney is believed by some to increase the susceptibility to pyelonephritis.

The correlation of two highly prevalent diseases with a wide range of diverse clinical and pathologic connotations accumulated in the categories of "pyelonephritis" and "high blood pressure" is a priori bound to be of a complex nature. The difficulty in untangling the etiologic interrelationships between these two diseases is affected also by the regular finding that a large proportion of persons with pyelonephritis have unknown antecedent urinary infections and or blood pressure elevation. In addition, pyelonephritis and/or urinary tract infections are at times superimposed upon other renal disease or anomalies. These factors account for the poor clinical recognition of pyelonephritis as a prominent cause of Bright's disease despite its ample and repeated demonstration in this role for nearly a century.

* From the Research and Educational Hospitals and Department of Medicine, University of Illinois College of Medicine, Chicago, Illinois. This investigation was supported in part by a Research Grant (E 1940) from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, United States Public Health Service.

TABLE II. RELATIONSHIP BETWEEN KINDS AND NUMBERS OF BACTERIA IN THE URINE

Bacterial Strains	Origin of Specimens: Female Catheter	Female Voided	Male Voided	All Specimens
Gram-positive	60 (47%)	205 (73%)	100 (85%)	365 (69%)
Per cent of isolates at 100,000/ml	5	4	2	4
Gram-negative	69 (53%)	76 (27%)	17 (15%)	162 (31%)
Per cent of isolates at 100,000/ml	70	40	11	46
All strains	129	281	117	527
Per cent of isolates at 100,000/ml	40	14	3	17

voided specimens, only 11 to 40 per cent were present in significant numbers All together, 17 per cent of 527 isolates, 3 per cent among males and 22 per cent among females, were present at the level of 100,000 or more organisms per milliliter of urine.

If judged upon the basis of persistent infection, the reliability of a single urine specimen with 100,000 or more bacteria per milliliter is highly accurate in reflecting the status of the patient, as shown in Table III. Pale-cell pyuria also usually occurred concomitantly with significant bacteriuria If pale-cell pyuria was present in a catheter specimen, persistent infection was observed in 88 per cent of the subsequent specimens, and in voided specimens from females, in 77 per cent. The presence of pale-cell pyuria with an insignificant bacterial count correctly anticipated

TABLE III. RELIABILITY OF URINE ABNORMALITIES IN A SINGLE URINE SPECIMEN FROM PATIENTS WITH HYPERTENSION AS DETERMINED BY A STUDY OF SERIAL SPECIMENS AT MONTHLY INTERVALS

	Number of Observations	Indication of Persistent Infection — Quantitative Urine Culture: Accurate Per Cent	False Positive Per Cent	Pale-Cell Pyuria: Accurate Per Cent	False Negative Per Cent	Disagreement Between Quantitative Bacterial Culture and Pale-Cell Pyuria Per Cent
FEMALES						
Catheter specimens	239	95	5	88	12	6
Voided specimens	177	84	16	77	13	12
MALES						
Voided specimens	155	100	None observed	97	None observed	3

the subsequent observation of significant bacteriuria in two-thirds of such cases. If both significant bacteriuria and pale-cell pyuria were present, a single urine specimen was 94 per cent predictive of the findings in serial specimens. Pale-cell pyuria was not indicative of infection if accompanied by a significant number of red blood cells and/or granular casts Both of these formed elements were notably absent in the urine sediment of patients with pyelonephritis, whether or not they had hypertension. Microscopic hematuria was observed with great regularity, however, among patients with grade III or IV hypertension.

Applying the criteria of significant bacteriuria and pale-cell pyuria, 12 patients with hypertension, two males and ten females, were found to have an active urinary tract infection All of these were caused by a single bacterial strain *Escherichia coli* was at fault in nine, *Aerobacter aerogenes* in two, and enterococcus species in one patient. Three-fourths of these patients, or 10 per cent of the entire group, were free from symptoms and had clinically unsuspected active chronic urinary tract infection Six of these patients have been shown by renal biopsy or subsequent autopsy to have had histologically significant pyelonephritis

Among the 90 patients, 25 had a convincing history of previous attacks of symptomatic urinary tract infections In this group, 12 had evidence of renal disease by pyelography, kidney biopsy, nephrectomy, autopsy, or persistent active infection This is a significantly greater number than the 15 such persons among the other 65 patients, in whom no history of antecedent urinary infection was obtained Three patients had nonbacterial renal disease, two with chronic glomerulonephritis, and one with renal amyloidosis In summary, if one includes radiographic or histologic evidence of previous pyelonephritis and patients with asymptomatic active urinary tract infections during the period of observation, 19 per cent of males and 32 per cent of females with arterial hypertension had pyelonephritis at some time past or present. This maximal clinical incidence of pyelonephritis corresponds well with the observed frequency of pyelonephritis among patients with hypertension at autopsy Active infection was found in 12 patients, or 13 per cent of the group (Table IV, line 1), in comparison with other groups of patients to be discussed.

In 327 patient months of observation among 53 females and in 118 patient months of observation among 37 males, all with hypertensive disease, acquisition of a transient or chronic urinary tract infection was observed in 6 hypertensive patients These infections occurred with the same frequency among males and females

Patients with either active infection or inactive pyelonephritis were a larger proportion of the age group under 50 (32 per cent) than of the group over 50 years (19 per cent) On the other hand, they were dis-

Severe pyelonephritis was distinctly more frequent than chronic glomerulonephritis, which was present in 70 cases of intercapillary glomerulosclerosis, which was found in 43 cases. Furthermore, 10 per cent of patients with arteriolar nephrosclerosis, 12 per cent of those with chronic glomerulonephritis, and 30 per cent of those with intercapillary glomerulosclerosis had chronic pyelonephritis in addition.

Among the autopsy series, 124 cases classified as nonobstructive chronic pyelonephritis were suitable for analysis of the blood pressure relationship. Excepting uremia, the most prevalent clinical condition associated with pyelonephritis was hypertension (35 patients). Azotemia of marked degree (NPN over 100 mg. per cent) was present in 29 per cent of the cases in this group, and azotemia of minor degree (NPN over 50 mg. per cent) in 50 per cent. Hyperparathyroidism, presumably secondary to chronic uremia, was present in 9 of these patients. Other diseases associated with chronic nonobstructive pyelonephritis, in descending order of frequency, were diabetes mellitus (15 patients), hepatic cirrhosis (11 patients), nephrolithiasis (10 patients), neoplasms of the gastrointestinal or genital tract, exclusive of the urinary tract (13 patients), rheumatic heart disease and other collagen diseases (6 patients), and ulcerative colitis (5 patients).

Death was attributed to acute pyelonephritis in 6 patients, to chronic pyelonephritis and uremia in 28. Cardiovascular disease was the major terminal illness in 20 cases, with chronic pyelonephritis considered a contributing cause of death in 4. Thus, chronic nonobstructive pyelonephritis was the direct cause of death in about one-third of patients with that illness, and hypertensive cardiovascular disease caused demise in one-fifth Approximately one-half of these patients died from renovascular illness.

Among the patients classified as having obstructive chronic pyelonephritis, 59 were suitable for analysis for associated hypertension. The causes of obstruction were benign prostatic hypertrophy (17 patients), carcinoma of the bladder (10 patients), carcinoma of the prostate (7 patients), neuropathic bladder (8 patients), carcinoma of the female genital organs (4 patients) or gastrointestinal tract (5 patients), and infrequent miscellaneous conditions. In addition, 12 of the patients had nephrolithiasis.

One-half of the p[illegible] ith obstructive [illegible] e chronic
pyelonephritis at au[illegible] cultures [illegible] a few weeks to
11 years before [illegible] V). Signific[illegible] were found in
75 per cent of cases [illegible] structive c[illegible] phritis and in
83 per cent of [illegible] obstruct[illegible] in Table VI,
[illegible] hia coli and [illegible] genes [illegible] ma[illegible], of
[illegible] isolates, [illegible] cent [illegible]
[illegible] in si [illegible]

TABLE V. Initial Recorded Urine Culture from Patients with Chronic Pyelonephritis at Autopsy

Time of Culture Before Death	Number of Observations of Patients with Significant Bacteriuria*	Per Cent of Cases with Demonstrable Active Infection
1–6 days	10/19	53
1–3 weeks	18/23	78
1–5 months	17/26	65
½–2 years	14/19	74
3–11 years	7/8	88
Total	66/95	70

* 100,000 or more organisms per milliliter of urine

the positive cultures from nonobstructed cases and in almost one-half of those with obstruction. The bacteriologic results from hypertensive patients in the group did not differ from the results from the normotensives

For the purpose of further analysis, only patients with records of renal weights, body weight, and body length were considered. Among the [illegible] showed remarkable fluctuation of the blood pressure within the hypertensive range. Intermittent blood pressure elevations to hypertensive levels or only high systolic blood pressures were observed in an additional 9 patients.

The mean age for this entire group was 53 years. There were 57 males and 62 females The mean age for patients with all grades of hypertension was 49 years Severe hypertension (grade III or IV) occurred at a younger mean age, 41, and males predominated 12:9. Among the cases with grade I or II hypertension, the ratio of males to females was 8:14 and their mean age was 55 years.

TABLE VI. Bacteria Recovered from Initial Positive Urine Cultures of Patients with Chronic Pyelonephritis at Autopsy

	Number of Isolates	Per Cent of Positive Cultures
Escherichia coli	21	42
Aerobacter aerogenes	13	26
Other gram-negative species	8	16
Gram positive species	8	16

TABLE VIII. Historical, Clinical and Autopsy Data on 40 Selected Patients with Severe Pyelonephritis and/or Severe Hypertension

			HISTORY		URINARY TRACT INFECTION		
Case	Age	Sex	Familial Hyper-tension	Years Since First Elevation of Blood Pressure	Years Since First Symptoms of Urinary Infection	Months Since First Recorded Significant Bacteriuria	First Known Species Recovered from the Urine
GROUP I	MODERATE OR SEVERE PYELONEPHRITIS AND SEVERE HYPERTENSION						
1	52	M	Unknown	Unknown	49	<1	Hem enterococci
2	29	M	No	Unknown	12	4	*S aureus*
3	54	F	No	20	20	12	*E coli*
4	39	F	Yes	<1	None	6	*E coli*
5	52	M	No	8	20	12	*A aerogenes*
6	46	F	No	6	<1	<1	Multiple
7	22	M	Unknown	2	<1	<1	Coliform
8	44	F	No	2	8	1	Multiple
9	40	F	No	7	13	3	None
10	41	F	Unknown	8	30	96	*A aerogenes*
11	43	F	No	24	24	2	*A. aerogenes*
12	43	M	Equivocal	3	3	<1	None
13	21	M	No	5	10	18	Coliform
14	44	M	Yes	2	None	2	None
15	53	M	Equivocal	3	4	1	Multiple
16	47	F	No	7	Unknown	Unknown	Unknown
17	60	M	No	Unknown	Unknown	Unknown	Unknown
18	48	M	Unknown	4	12	96	*S aureus*
GROUP II	SEVERE HYPERTENSION AND MILD PYELONEPHRITIS						
19	40	F	No	5	None	6	None
20	26	M	No	Unknown	None	Unknown	Unknown
21	29	F	No	13	11	60	Coliform
22	37	F	Equivocal	17	1	15	*A aerogenes*
23	53	F	Unknown	2	None	2	None
24	50	M	No	15	None	Unknown	Unknown
GROUP III	SEVERE PYELONEPHRITIS WITH MARKEDLY REDUCED TOTAL RENAL MASS AND NO OR MODERATE HYPERTENSION						
	54	F	Yes	17	17	<1	Enterococci
	63	F	No	Unknown	None	<1	*E coli*
	69	F	Unknown	Unknown	<1	<1	Enterococci
	47	M	No		None	<1	None
	75	F	Equivocal	Unknown	34	Unknown	Unknown
	43	F	Unknown	3	None	Unknown	Unknown
31	75	F	Yes	Unknown	43	Unknown	Unknown
32	18	F	No	Unknown	1	10	None
33	16	F	No		None	1	None
34	6	F	No	Unknown	5	24	None
35	9	F	No	1½		15	Coliform
36	54	F	No	Unknown	None	60	*A aerogenes*
37	62	M	Unknown	Unknown	11	2	*Proteus*
38	47	M	No	Unknown	(27)	<1	Micrococci
39	43	M	Unknown	19	(40)	Unknown	Unknown
40	27	M	Unknown		(21)	Unknown	Unknown

YPERTENSIVE DISEASE		AUTOPSY DATA				INTERPRETATION
Months of Recorded Blood Pressure	Terminal Illness	Heart Weight in Grams	Renal Weight in Grams Right	Renal Weight in Grams Left	Renal Mass per Square Meter of Body Surface Area	Likely Origin of Hypertension
1	Uremia	Unknown	70	60	70	Renoprival
77	Uremia	500	72	68	77	Renoprival
24	Uremia, cerebral hemorrhage	495	70	60	78	Renoprival
7	Heart failure	590	110	115	143	Essential
20	Aortic rupture	480	120	50	103	Renoprival
<1	Uremia	640	110	160	130	Indeterminate
24	Uremia	470	70	19	47	Indeterminate
2	Uremia	550	95	0	55	Renoprival
16	Uremia	670	115	115	118	Indeterminate
96	Uremia	320	0	180	107	Renoprival
168	Cerebral hemorrhage	420	160	80	159	Indeterminate
1	Uremia	470	150	150	196	Essential
20	Uremia	540	130	120	160	Renoprival
14	Heart failure	930	170	150	203	Essential
21	Myocardial infarct	530	250	80	147	Indeterminate
72	Carcinoma	330	150	140	223	Essential
5	Myocardial infarct	400	80	110	109	Indeterminate
28	Uremia	750	97	97	90	Renoprival
60	Heart failure	580	200	190	229	Essential
6	Uremia	340	160	160	194	Essential
64	Cerebral hemorrhage	360	120	120	143	Essential
28	Uremia	645	110	105	134	Essential
2	Heart failure	550	120	170	174	Essential
24	Uremia	795	190	170	198	Essential
24	Heart failure	400	70	100	96	Renoprival
17	Uremia	290	55	50	67	Renoprival
<1	Uremia	320	16	70	86	Renoprival
<1	Uremia	440	65	60	81	None
132	Myocardial infarct	600	0	130	92	Renoprival
3	Carcinoma	510	50	80	80	Renoprival
2	Heart failure	410	165	0	101	Indeterminate
12	Uremia	300	80	70	120	Renoprival
<1	Uremia	275	70	90	103	None
20	Uremia	142	31	29	83	Renoprival
1	Uremia	210	42	25	69	Renoprival
120	Uremia	480	47	55	55	None
24	Uremia	500	60	60	72	Renoprival
72	Uremia	480	70	70	70	Renoprival
36	Uremia	710	101	11	63	Renoprival
<1	Uremia	360	80	120	120	None

In summary of the autopsy series, 60 of the 170 cases, or 35 per cent of those reviewed with pyelonephritis at autopsy, had associated clinical hypertension. It was of grade III or IV severity in 24 cases, in 6 of which the mild chronic pyelonephritis seemed to be causally unrelated to the hypertensive disease. Of the remaining 18 cases with severe hypertension and chronic pyelonephritis, chronic pyelonephritis could be inferred on the basis of clinical history and a severe reduction in renal mass to have been the cause of hypertension in one-half, or 38 per cent of all cases with grade III or IV hypertension and pyelonephritis. In the other 9 cases, 38 per cent of those with severe hypertension, severe pyelonephritis was coexistent without a marked reduction in renal weight, and its influence upon the blood pressure could not be assessed. Among the cases with less severe hypertension, grades I and II, a severe reduction of total renal mass was more prevalent than among the group with grade III or IV hypertension. In 55 per cent of the patients with nonobstructive chronic pyelonephritis, this disease could be inferred to have been the cause of grade I or II hypertension.

Comments

The reliability of quantitative urine cultures and pale-cell pyuria for the diagnosis of chronic urinary tract infections confirms our previous experience and that of others.[17, 24 26, 33, 38, 39, 43] Among significant urinary isolates gram-negative species, in particular *E. coli*, were highly prevalent. Gram-positive organisms, on the other hand, were almost always contaminants. This conclusion was reached because they were not consistently isolated in serial cultures, pyuria was not present in the urine from which they were recovered, and also because they were particularly prevalent in voided specimens. This also applies to their occurrence in mixed cultures.

The predominance of *E. coli* in the urinary tract has already been observed by Escherich[47] and has since been re-emphasized by many others.[5, 8, 20] This species was found as the etiologic agent in 75 per cent of unsuspected urinary tract infections among patients with hypertension. Among the patients who had pyelonephritis at autopsy, it also was the prominent species. These findings suggested the bowel as a source of infection.[20, 21] The occurrence of gastrointestinal disease, including neoplasms, ulcerative colitis, or cirrhosis of the liver, in approximately 20 per cent of cases with pyelonephritis at autopsy is therefore of interest. Bypass of the portal system of the liver in cirrhosis, with shunting of blood directly from the gastrointestinal tract into the systemic circulation, might enhance the incidence and severity of bacteremia arising from

the intestinal tract. Also the frequency of pelvic surgery among female patients with pyelonephritis was conspicuous and has been noted by others.[20]

The search for pyelonephritis among the hypertensive clinic patients yielded an incidence comparable to that in most autopsy series of hypertensive patients This frequency is higher than the 11.6 per cent recently observed among the routine autopsies in our hospital, and the latter rate has not changed greatly in 33 years[23] Active infections among the hypertensive clinic patients also were more frequent than among other outpatients with chronic respiratory infections The incidence of significant bacteriuria among the latter group is not higher than that observed for a general outpatient population,[27] and thus the bronchi do not appear to be important as a potential source of urinary tract infection in adults. This is noteworthy since another chronic infection, pyoderma, has been incriminated as a frequent focus of urinary tract infections in infants and children.[49]

Bladder catheterization as a potential source of iatrogenic urinary tract infections has been recently re-emphasized by Beeson.[5] Among our female hypertensive clinic patients, diagnostic catheterization could perhaps have caused chronic infection in 3 per cent, but this represents only two infections in more than 250 procedures These cases were excluded from consideration in the analysis reported here. The acquisition rate of infections during the study period was not greater in females than in males who were never catheterized. Therefore, diagnostic catheterization per se did not appear to be an important cause of infection in this group.

In some of the clinic patients, urinary tract infection without demonstrable renal involvement appeared to be incidental to essential hypertension. In others, pyelonephritis was clearly superimposed on the nephrosclerosis of essential hypertension. Animal experiments[3, 9, 45, 52] suggest that the vascular changes in these kidneys with ensuing renal scar formation make them more susceptible to infection In 2949 autopsies, however, the over-all incidence of pyelonephritis among patients with nephrosclerosis was of the same order as among the patients with glomerulonephritis and the average for all autopsies.

Under the influence of Goldblatt's classic experiment,[15] there was a shift of clinical emphasis to unilateral renal disease as the basis of hypertension from pyelonephritis Weiss and Parker[50, 51] and more recently Kincaid-Smith[21] and others[25, 33] proposed that ischemia from inflammatory endarteritis caused the hypertension in chronic pyelonephritis. Fanconi and co-workers[13] have suggested an "inflammatory irritation of the renin producing cells of the distal nephron" as an alternative hypothesis.

Pyelonephritis is the commonest lesion in hypertensive unilateral renal disease as well as in hypertension that has been cured by nephrectomy[44]

making the autopsy reports available to us, and to Dr. C. Johnston for his cooperation and assistance in the Hypertensive Clinic.

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Materials and Methods

For the present study it was elected to attempt to identify the sites and types of renal damage in pyelonephritic kidneys that are most closely correlated with the presence of hypertension, using the methods of morphologic pathology. Previously, persons whose kidney biopsies showed pyelonephritis were found to have *significantly higher average diastolic* blood pressures and a worse prognosis for the same grade of arteriolar nephrosclerosis than patients with essential hypertension.[20]

The structural composition of surgically removed human kidneys was investigated in a series of 38 cases divided into four major groups, which comprised (*a*) 10 "normal" kidneys removed from normotensive patients because of renal hemorrhage, cyst, hydronephrosis, calculus, or carcinoma, (*b*) *19 chronic pyelonephritic kidneys, including 9 removed from patients* with persistent hypertension at levels of 150/90 mm. Hg or more and 10 from patients without hypertension; (*c*) 9 kidneys without pyelonephritis that had been removed for the treatment of a hypertension believed due to unilateral kidney disease, or so-called Goldblatt kidney.[4] Two of the 9 pyelonephritic kidneys from hypertensive patients had been removed for this reason. (*d*) Five Goldblatt-type cases had contralateral *renal biopsies studied The operative material was mostly from* Dr. Reginald H. Smithwick, Dr. William J. Porell and associates, Massachusetts Memorial Hospitals, Boston

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The weight range of the "normal" kidneys was 118 to 190 Gm. in 8 suitable cases, average 155 ± 22 Gm. For 5 pyelonephritic kidneys that were weighed without the perirenal fat, the weight range was 58 to 210 Gm, average 138 ± 62 Gm Ten so-called Goldblatt kidneys weighed *from 20 to 188 Gm*, average 92 ± 49 Gm

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colored pencils (Figure 1). Ten sheets of paper representing random fields of kidney cortex were mapped from each case. The approximate total area mapped was 6.6 sq. mm. per case. Thereafter, the paper sheets were cut along the lines and each kidney component was separated, weighed, and counted. The weights of the pieces of paper were converted

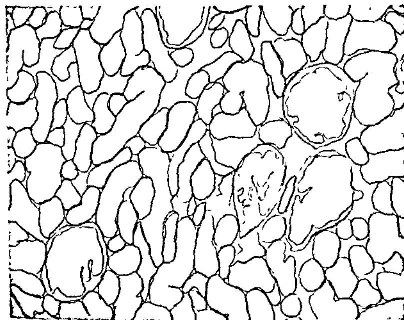


FIGURE 1. One-tenth of the kidney map made from a histologically normal portion of kidney, reduced from a magnification of about ×600. In the original the glomeruli were outlined in purple, proximal convoluted tubules in red, distal convoluted tubules in blue, atrophic tubules when present in orange, arteries in black and veins in green, to permit differential weights and counts.

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Microscopic sections of the 38 kidneys and 5 renal biopsies stained with hematoxylin and eosin or phosphotungstic acid were projected onto sheets of paper measuring 12 by 9½ inches, at constant magnifications of about ×600. Major histologic renal components, comprising the glomeruli, the proximal, distal convoluted and atrophic tubules, the arterial and venous blood vessels, and the interstitial tissues including scars and inflammatory foci were drawn in outline on the paper, each with different

colored pencils (Figure 1). Ten sheets of paper representing random fields of kidney cortex were mapped from each case. The approximate total area mapped was 6.6 sq. mm. per case. Thereafter, the paper sheets were cut along the lines and each kidney component was separated, weighed, and counted The weights of the pieces of paper were converted

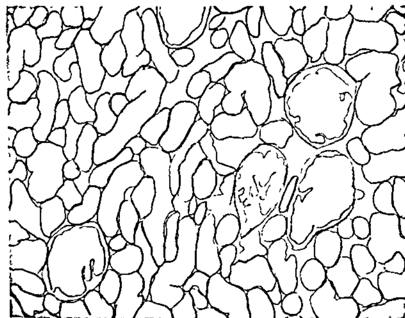


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cantly decreased only in the group with pyelonephritis and hypertension This was not true of pyelonephritis without hypertension. There were no significant variations in the percentages of the total areas of all tubular tissue among any of the groups Too few Goldblatt kidneys with pyelonephritis were available for a valid statistical comparison, but in those studied the same decrease of proximal convoluted tubules and increase of interstitial tissue were found as in the other pyelonephritis cases with hypertension.

While not statistically significant, it is of interest that the percentage area occupied by vessels had the smallest value in the group of pyelonephritis cases with hypertension. The mapping method now needs to be applied more specifically to study this aspect of pyelonephritis, which was emphasized by Kincaid-Smith.[11] She found extreme internal renal vascular narrowing, a process which Weiss and Parker[23] had termed hyperplastic arteriolosclerosis, to be common in autopsied hypertensive pyelonephritis cases.

No significant difference was found in the sizes, expressed for convenience as the average diameters, of glomeruli or renal tubules in any of the other groups. As a method of recognizing the so-called Goldblatt kidney histologically, a diminution in sizes of the glomeruli and tubules proved useful only in one of the eleven mapped cases[10] This kidney weighed 60 Gm with an average glomerular diameter of only 104 microns, of proximal tubules 31 microns, and of distal tubules 32 microns. There was no postoperative fall of the blood pressure, which remained at levels of 290/120 mm. Hg.

Comparison of the numbers of mapped proximal convoluted tubule cross sections showed a borderline or dubious statistically significant decrease, compared with "normal" kidneys, only in the group with pyelonephritis and hypertension, $t = 2.67$, $p < 0.02$. The decreased total area of proximal tubules was thought ascribable mostly to the transformation of some proximal tubules into atrophic tubules, and to a lesser extent to decreased diameters and numbers of the individual proximal convoluted tubules.

The indices of granularity of the juxtaglomerular cells (JGI) were fairly consistent in normal and pyelonephritic kidneys, but they showed wide variations in the Goldblatt cases There was generally an increased value of the JGI in Goldblatt cases, but this did not reflect the presence of large, clear, nongranulated, apparently hypertrophied cells, often found in the most acute cases of unilateral renal disease with hypertension, which also included the best therapeutic results after nephrectomy. When the contralateral kidney biopsies were available, their JGI were comparatively decreased. In one case, with atrophy of the upper third of the kidney, the remaining two-thirds appeared normal. The respective indices were

TABLE II COMPARISON OF JUXTAGLOMERULAR CELL COUNTS

	Cases	Total Cells	Types II-III	Per Cent Types II-III
JGCC, Based on 25 Juxtaglomerular Apparatuses				
Normal	10	192 ± 21	9 ± 7	4 7
Pyelonephritis, no hypertension	10	192 ± 34	10 ± 7	5 2
Pyelonephritis and hypertension	8	189 ± 33	16 ± 10	8 5
Goldblatt	14	260 ± 59	26 ± 16	10 0
Contralateral biopsy	4	220 ± 38	15 ± 10	6 8
Relation of JGCC to Therapeutic Effect of Removal of Goldblatt Kidney				
Excellent	3	324 ± 33	23 ± 12	7 1
Intermediate	4	249 ± 68	21 ± 11	8 4
Poor	2	226 ± 11	48 ± 10	21 2

76 for the atrophic part, 50 for the midportion, and 30 for the lower third These results require further investigation.[2, 3]

Counts of juxtaglomerular apparatus cells, compared for different groups, are given in Table II No statistically significant differences in the JGCC were found, except when therapeutically successful cases of Goldblatt kidney removal were compared with the "normal" group In this



FIGURE 3 Hypertrophy a[illegible] case of unilateral renal d[illegible] result following [illegible]

FIGURE 4 Normal appeara[illegible] juxtaglomerular apparatus from a histologically normal kidney, for comparison with Figure 3 S55-2409, Bowie stain, ×680

and proximal tubular changes differed from those of Goldblatt kidneys Consequently, the high blood pressure that complicated chronic pyelonephritis was believed most likely to represent an impure or mixed form of secondary renal hypertension. If all or most of the Bowie-stained granules in the kidney represented vasopressor material, such as renin, the smaller amounts, but more strategically located, would be in the renal juxtaglomerular cells and other perivascular foci, but the larger and perhaps *more significant stores of vasopressor material would occur in* the cytoplasm of cells of the partly degenerated convoluted tubules

The interpretation that has been attempted is intended merely as a unifying explanation, useful in suggesting further investigations into an unsolved problem both of theoretical and practical interest.

Summary

Thirty-eight surgically removed human kidneys, including 19 with chronic pyelonephritis, were mapped and analyzed for their individual histologic components, in order to study pathologically the relationship between pyelonephritis and hypertension. Counts and indices of the juxtaglomerular cells were computed from these and an additional 4 cases. Salient findings in pyelonephritic kidneys from hypertensive patients were statistically significant increases in the percentage area of interstitial tissue, and significant decreases of the percentage area and perhaps in the number of proximal convoluted tubules, when compared with control groups. Renal cortical vascularity might have been decreased. The heavily granulated juxtaglomerular cells were relatively increased in cases of pyelonephritis accompanied by hypertension. The changes observed are interpreted to favor secondary renal hypertension developing as a complication of pyelonephritis

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according to clearly defined criteria, have renal lesions which are probably the initiating mechanisms of their hypertension. Further, these lesions and the hypertension are often *correctable by nephrectomy or* renal vascular surgery. So the picture has been changed in the past four years, we have gone the complete circle from the early days of the place of the urologist in the sun to his complete eclipse and now back again to a more defined and limited, but vital, role. I cannot help expressing admiration for their "carnation-in-the-buttonhole" tenacity.

The Initiation of Renal Hypertension

The fact that hypertension could be elicited by a clamp constricting the renal artery led almost inevitably to the thought that lack of blood, or "ischemia," was the immediate cause of the hypertension. The term "renal ischemic hypertension" is still widely used but without objective evidence to support the concept behind it. Proof that renal ischemia is necessary to elicit hypertension has never been found. Since renal blood *flow may be normal in early essential hypertension, some investigators* assumed that experimental renal hypertension produced by compression of the renal arteries or of the renal parenchyma had little in common with *the human variety.*

The nature of this problem was delineated by the demonstration of Corcoran and Page [4, 5] that chronic experimental renal hypertension produced by a clamp can occur without ischemia as measured by clearance techniques, no correlation was found between mean blood pressure levels and rate of renal blood flow. Warthin and Thomas [41] also found no permanent reduction in renal blood flow as measured by phenol red clearance after application of a Goldblatt clamp. Angiograms made two to six weeks after clamping the renal artery showed no reduction of renal blood flow in dogs and rabbits.[7]

It is not usually realized how severely a blood vessel must be constricted before appreciable fall in flow occurs, especially when blood pressure increases concurrently. Further, it has not been generally rec*ognized that when a metal clamp is put on a blood vessel* the wall within the confines of the clamp becomes much thinned and stretched. The blood vessel wall thus occupies less of the clamp space than initially and much more blood can flow through it. To avoid the uncertainty of changing flow through the clamped area of the renal artery, Corcoran and Page[5] used Cellophane perinephritis to elicit hypertension. Since this requires weeks and avoids the uncertainty of the degree to which the caliber of the renal artery was reduced by the clamp, it was much simpler to show that blood pressure rose without concurrent production of renal ischemia.

Another approach to this problem was the production of coarctation of the abdominal aorta by clamping just above the renal vessels Since the aorta is a large vessel, the degree of narrowing is more easily controlled Friedman, Sugarman, and Selzer [10] found after a period of 14 days during which hypertension developed that reduction of both mean and pulse pressure was followed by dilatation of the renal vessels distal to the clamp. If the decrease in pressure was not too great as a result of constriction of the aorta, renal ischemia did not result. The initial period of reduced renal artery pressure may stimulate the release of a blood pressure raising material which tends not only to maintain arterial pressure at normal levels but to raise it to hypertensive ones as well. Hawthorne, Perry, and Pogue [12] reduced femoral arterial pulse pressure by a clamp on the aorta without concurrently reducing mean pressure and noted a significant rise in mean femoral pressure two days later. Earlier it had been shown by Kohlstaedt and Page [15] that renin was liberated from a perfused dog's kidney when pulse pressure was reduced but mean arterial pressure and renal blood flow were maintained constant by a pump. This again suggests reduction in pulse pressure as the stimulus for development of experimental renal hypertension This type of obstruction with resultant renal hemodynamic changes may well have its counterpart in the congenital coarctation of the abdominal aorta with resultant renal hypertension described by Fisher and Corcoran [8]

The Nature of the Renal Pressor System

[illegible]
by [illegible]
m [illegible]
this case seems to be angiotensin

I need not detail all the physiologic evidence supporting this view It is strong Alternate explanations have been given, chief among which is that the kidneys act to destroy pressor amines which might be generated elsewhere Still a third view is that the kidneys secrete a substance into the blood stream which keeps blood pressure normal. Lastly, there is the hypothesis that the level of blood pressure is controlled by a renotrophin

Braun-Menendez [3] considered the size and function of the kidneys to be regulated by a hypothetical renotrophic by-product of protein metabolism contained in blood The rate of production of this renotrophin was increased by hormones of the pituitary, thyroid, and testes as well as protein-rich diets Its blood levels would depend on the equilibrium between rate of formation and its destruction by the kidneys. An increase in renotrophin production causes renal hypertrophy and hyper-

recently been purified. So far as is now known, it has no pharmacologic properties except those of angiotensin-2, but to a much smaller degree.

The blood contains an enzyme discovered by Skeggs, Kahn, and Shumway [36] which converts angiotensin-1 to angiotensin-2 by splitting off histidyl-leucine. The eight-membered peptide is the most active pressor agent known. It also has strong oxytocic activity. It is this substance that is believed to cause renal hypertension. Unfortunately this has not yet been given rigid proof, for several reasons.

One of the chief is that, being a peptide, there are no currently available methods for measurement of its levels in the blood except relatively crude biologic ones Another is that angiotensin-2 is unstable in that it is readily destroyed by several proteolytic enzymes that are widely distributed in the body. Still another is that almost nothing is known about how angiotensin acts. It certainly has a direct action on smooth muscle but it may also act indirectly by retention of salt.

Work on angiotensin has been seriously hampered by lack of pure material. Although we discovered this substance 20 years ago, it was not until a year ago that pure material was available for investigation.

Elliott and Peart [8] in 1956 showed the amino acid sequence of the decapeptide, that is, angiotensin-1, and Skeggs *et al.*[34] found that angiotensin-2 was a chain of eight amino acids identical with angiotensin-1 but with two amino acids lost from one end. The final proof of structure, however, comes only with synthesis.

Our group (Schwarz, Bumpus, and Page [35]) performed this synthesis starting with the eight naturally occurring amino acids, through 26 steps to the final angiotensin-2 The synthetic material proved identical with the natural. A somewhat similar synthesis was performed at the Ciba laboratories by a team headed by Schwyzer.[33]

It is now clear that the body employs a very complex system to achieve the liberation of angiotensin This may be its device to store the inactive precursors and at the same time to insure close regulation of its production and release. There are many steps at which the reaction could be slowed, speeded, or stopped.

An interesting fact is that at least six substances acting on smooth muscle are peptides and can be prepared from the same alpha-2 globulin fraction of blood. These are angiotensin, pepsitocin, pepsitensin, pepsanurin, kallidin, and bradykinin. In many ways these peptides are similar to the pituitary's oxytocin and vasopressin. These are also octapeptides but of different structure. It would be expected that the body employ proteolytic enzymes other than renin to liberate this Pandora's box of smooth muscle stimulants.

Whenever there are stimulants there are inhibitors and these we are only

now beginning dimly to discern From the therapeutic point of view the inhibitors may well constitute the "practical" side of the problem Our own evidence suggests their presence in the kidney and in the blood and that they are of protein nature.

FIGURE 1. Structure of synthetic angiotensin, showing the splitting off of histadyl leucine from the inactive decapeptide to form the highly active octapeptide

Another interesting possibility exists for the regulation of smooth muscle activity in what is known as transpeptidation. Intracellular proteinase not only catalyzes the hydrolysis of peptides but it also catalyzes replacement reactions Transpeptidation is an example of the latter. It appears that an activated enzyme-substrate complex can interact with water, resulting either in hydrolysis, or with a replacement agent, resulting in transpeptidation. Since pH strongly determines the extent of either, it has been suggested that in physiologic ranges replacement reactions may far outweigh the hydrolytic reaction which occurs at more acid pH

The possibility thus exists for interconversion or building still more complex peptides by using the catalyzing action of the intracellular proteinases. While these mechanisms have not been proved to be physiologic ones, the chain of coincidences makes me believe that these possibilities should not be overlooked.

High degrees of structural specificity have come to be regarded as a hallmark of proteins. This is evident in the exquisite specificity of immune reactions. Yet among the peptides, as pointed out by Woolley and Merrifield,[42] rigorous specificity does not seem to hold for many of their known physiologic functions. Oxytocin and angiotensin illustrate this beautifully Both contain eight amino acids but they are different ones and are arranged quite differently, yet both substances seem capable of subserving the same function.

Is Angiotensin the Humor of the Kidney?

In brief, I think it a fair judgment that angiotensin is by far the most likely pressor agent to be the cause of renal hypertension. Although several attempts have been made to measure it in blood, none has given an unequivocal answer. So much blood is required for some of the methods of measurement that other pressor agents such as adrenalin and noradrenalin are secreted into the blood as a reaction to blood withdrawal. The isolation and partial purification involves many steps and the final product has had to be measured biologically.

Gollan, Richardson, and Goldblatt,[12] using one of these complex methods, found that a pressor substance was present in large amounts in 200 ml samples of blood from dogs with malignant hypertension or with experimental renal hypertension up to 3 months duration. The pressor substance was destroyed by angiotensinase and had other physiologic and chemical properties of angiotensin. The claim of the finding of "large amounts" of pressor substance should have settled the problem in 1948. More than 10 years later there still is no confirmation and it seems unlikely there will be.

Kahn *et al.*[17] assayed in anesthetized rats 250 ml samples of blood of essential and malignant hypertensive patients after concentration and purification. Angiotensin was found greatly increased in malignant hypertension, while in essential hypertension there was overlap with the normotensive group although the mean concentration was twice normal. The amounts they recovered from the benign group were so small as to make it meaningless Kahn *et al*[16] also studied the dialysate of normal dogs using an artificial kidney and found a pressor substance which had all of the properties then known for angiotensin. The dialyzing process

may have caused it to appear in the blood, and hence in the dialysate, whereas in the normal group of animals they studied later it was found only in the minority of animals

A cross-circulation method has recently been developed by Blacquier *et al.*[1] in which a volume of blood equal to the total blood volume can be exchanged within 15 minutes When angiotensin was infused into one of the pair of rats, the pressure of the other animal rose rapidly when cross circulation was commenced. Rats with long-standing renal hypertension failed to show this

Recently Braun-Menendez and his group[20] have described another method for assay in 50 ml. of arterial blood The recovery rate of angiotensin is high. The results and quantitative evaluation of this method will be awaited with interest

What is clear is that after 15 years, using current chemical and biologic methods, there is no convincing proof that renin or angiotensin participates in the genesis of hypertension. But it must be equally clear that there is overwhelming evidence that the problem of measurement is at the very core of the problem The answer to the question is too important to accept less than the best simply because it would be more comfortable to have it settled.

Evidence of a different sort has been presented by Wakerlin[40] He elicited neutralizing antibodies to dog's renin by repeated injections of hog's renin. The blood pressure of renal hypertensive dogs fell in direct proportion to the antirenin titer of the plasma High titer homologous antiserum could be shown by passive transfer to be antihypertensive in chronic renal hypertensive dogs. This constitutes strong evidence of the pathogenetic role of renin in experimental canine renal hypertension

Possible Secondary Mechanisms Maintaining Elevated Arterial Pressure

Thus far I have implied that the renal pressor system was almost the only mechanism for keeping blood pressure elevated in renal hypertensives This is certainly far from true, because the other half of the equation of blood pressure regulation is the state of the bodily substrate on which angiotensin must act. This is reflected in the concept of cardiovascular reactivity[28] which has been the subject of our inquiries for many years For convenience, this concept can be broken down into a number of aspects, the first of which is nervous control.

The response of the blood vessels and heart, as measured by stimulation by angiotensin and pressor amines, is greatly heightened, or "augmented," by denervation, whether by cutting the nerves or by blockade of ganglia

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it becomes inadequate for the other amino acids concerned There were further subtleties noted by Robson and Rose.[10] A similar lysine load to a cystinuric did not increase the output of the other three amino acids. This is what one would expect, for in cystinurics the clearances of all four amino acids are very high already, the tubular reabsorption mechanism being almost completely blocked.[1] On the other hand, a large load of glycine in a normal human did not appear to have any effect on the excretion of other amino acids. Most ingenious genetic evidence has been presented by Harris, Mittwoch, Robson, and Warren [11] which supports fully the idea of a specific enzyme defect in cystinuria and which has the special advantage of being completely independent of our other evidence. Why on earth should these four unlikely bedfellows (the amino acids, not Harris *et al*!) be so closely related in the renal tubule? This would well repay more serious study along obvious lines, meanwhile, the not very seriously intended theory of Dent and Rose [8] that the resemblance is due to a similar separation in space of the two basic groups in each molecule remains in the field unchallenged.

The practical value of such a specific amino acid excretion in a disease hardly needs stressing. The paper chromatographic pattern can be recognized at a glance — like someone's face The diagnosis can be made from a drop or two of urine without any need of more quantitative methods, and without any clinical or other examination of the patient.

Most of the other hereditary renal aminoacidurias, and all the acquired ones, are different from this. The pattern of amino acid excretion, while being grossly abnormal and still of great help in diagnosis, is less specific. When mild it involves mainly the smaller molecular weight amino acids occupying the central cluster of spots on the paper chromatograph. When more gross it also involves the other amino acids and the pattern begins to resemble that of normal plasma. Furthermore, the pattern is known in some cases (and suspected in most of the others) to vary considerably with extraneous factors. This suggests strongly that the renal tubule damage is of a more blundering nature, probably secondary to some other "toxic" factor and probably implicating many different enzymes and many different renal functions, glomerular as well as tubular. This can be well illustrated by considering the kidney in galactosemia.

Many workers have now noted the occurrence of renal tubular dysfunction in untreated cases of galactosemia which improved when they were treated with a galactose-free diet. There is a marked renal aminoaciduria, mainly affecting those of small molecular weight shown as a central cluster of spots on our standard chromatograms. Renal acidosis may also occur, we have unpublished data on a patient who also manifested renal glucosuria, and there may be proteinuria, which recently Butler and Flynn [3] showed to comprise mainly an unusual mixture of

globulins which they found characteristic of many other patients with renal tubular disorders. The pathogenesis of the tubule dysfunction was studied by Cusworth, Dent, and Flynn,[2] who were able to study a child of 2 years with galactosemia who had been given a galactose-free diet since 1 month of age. Renal function was quite normal at first. When given 20 Gm. a day of galactose for 15 days under otherwise very constant conditions, interesting urinary changes occurred. There was at once the appearance of a gross galactosuria which continued unchanged throughout the 15 days, but which rapidly stopped when the galactose was stopped. The expected aminoaciduria did not definitely appear for several days and was still increasing on the last day of the galactose, by which time it had become pretty gross. It then lessened slowly to become normal again about 7 days after the galactose had been stopped. Clearly the galactose was not immediately the cause of the aminoaciduria, in contrast to the relation between lysine and the other three amino acids in the experiments of Robson and Rose.[16] It is easier to imagine here a less specific "toxic" effect of the galactose whereby the tubule cell becomes incapable of normal metabolism and so becomes slowly depleted of ATP, which perhaps leads to the generalized disruption of metabolic activities which then is the cause of what we actually observe clinically and biochemically in these patients. That the cell dysfunction is entirely metabolic is suggested by its ready reversibility even in patients who have been undiagnosed and therefore untreated for a year or more. It is also obvious that such an aminoaciduria need not be specific for the disease in question, even when (as in galactosemia) the disease is known to be a true "inborn error of metabolism," due to a primary single enzyme defect. The observed phenomenon, the renal aminoaciduria, is removed many stages from the primary gene action. It will therefore vary with many factors such as the amount of galactose in the diet, with the age of the patient, and with his previous dietary history. Also it is not necessarily reversible since it may be associated with gross morphologic changes such as the atrophic proximal tubule in the Fanconi syndrome[4] and the increasing renal glomerular damage which always occurs in cystinosis. (There are no microscopic changes in the kidney in cystinuria.)

Another curious renal aminoaciduria is that occurring in classical vitamin-D-lack rickets.[11] This is quite gross in some cases but varies considerably in degree from case to case, presumably on account of the varying degrees of vitamin deficiency. In adults it is much less spectacular as far as our experience of only four cases goes. When vitamin D is given, the aminoaciduria disappears slowly — in 1 month in the only child case we have so far followed. This is just like galactosemia but with a slightly longer time scale. It is of interest that at least one very rare form of hereditary rickets (this does not go for most forms of "resistant rickets")

sodium, and potassium and for inability to make ammonia and to acidify the urine. We should look too for signs of renal glomerular damage and examine the urine proteins by electrophoresis in case they manifest the very characteristic "renal tubular" pattern (which does not occur in cystinuria and Hartnup's disease). When this more generalized renal damage is detected we know that it is probably secondary to some disorder elsewhere, and are thereby encouraged to look harder for it. The renal tubule may be considered to be the mirror of the metabolism of the whole organism and we may well rejoice in its unique accessibility for detailed study.

Perhaps I can illustrate this last point by referring to some recent unsuccessful work on the Fanconi syndrome. Dr. P. Randall was impressed with the resemblance between the renal tubule defects in this syndrome and those occurring after maleic acid intoxication.[12] He wondered if an inborn error of maleic acid metabolism might lead to high plasma and urine levels and therefore produce the observed renal functional abnormalities With help from Dr. F. C. Battaglia he worked out a sensitive method for maleic acid assay in urine and we then sent him urines from five patients with four different forms of the "Fanconi syndrome" Alas, no excess maleic acid was found. I believe, however, that this represents the correct attitude to this particular problem, and I am confident that it will pay dividends sooner or later. Indeed, the maleic acid hypothesis is not ruled out yet. There could still be an intracellular defect not revealing itself by analysis of body fluids

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detailed chart on the crucial patients, including a history of urinary tract infection and a history of known hypertension

DR FREEDMAN: Dr. Grieble, would you comment on the frequency of urinary tract instrumentation in the various groups of patients, and whether those with known hypertension, for example, might have been more likely to have had retrograde pyelography as part of the investigation for their disease, in contrast to the group with bronchiectasis.

Also, you gave us the sex incidence of your group of patients with essential hypertension, and I wonder what the sex incidence was in those who were found to have bacteriuria, and how this would compare in the three groups.

DR. STRAUSS May I add a question, Dr. Grieble. Isn't the incidence of hypertension of 7 per cent in the patients with bronchiectasis rather low for an unselected population? Of course, I don't know the ages involved

DR GRIEBLE Iatrogenic infection introduced by means of diagnostic catheterization was not a problem. The incidence of introduced infection was not higher than 2 or 3 per cent among the females who were catheterized at monthly intervals. This represented possibly two infections introduced by 250 diagnostic catheterizations and these two patients are excluded in the calculations

The different sex distribution among the bronchiectasis patients as compared with the hypertensive patients may play a role There was a 6 to 4 preponderance of males over females in the bronchiectasis group. In the hypertensive group the ratio was just about the reverse When we made the breakdown by sex, the groups were too small for any statistical conclusions. We do not feel, however, that the sex difference accounts for the difference in incidence of urinary tract infection

We did not test the incidence of hypertension among random groups of outpatients by our criteria. The 7 per cent was obtained from those patients who had had at least three blood pressure recordings at different times. If any of three or more such recordings were within the normal range the patients were not considered hypertensive This is probably the explanation of the lower than expected incidence of hypertension among the patients with bronchiectasis. These same criteria, however, were applied to all the outpatient groups studied.

DR PERKOFF: In our patients and in other patients with the hereditary forms of renal disease associated with nerve deafness amino acid pattern has been consistently normal I should like to ask Dr. Dent if he has

observed such patients in whom either primary or secondary amino-aciduria was a feature of the disease.

DR. DENT We haven't had such a case to study. We are greatly interested to read the literature, and wonder what was done It is very interesting to hear that you have done amino acid studies This is just the kind of disease in which one would expect aminoaciduria or other signs of renal tubular dysfunction

DR KARK: I enjoyed Dr. Sommers' ingenious and painstaking study. We all know that in unilateral renal artery disease removal of the kidney is followed by a normal blood pressure in nearly 100 per cent of the patients, whereas in 1000 cases of unilateral pyelonephritis with contracted kidney and hypertension, removal of these 1000 kidneys resulted in a normal blood pressure in only 20 per cent of the cases. Is it possible that we have two types of hypertension in these cases?

Dr Sommers has been studying the contraction of the scars on the kidneys. I wonder whether sometimes a scar might nip off a blood vessel and cause renal artery hypertension in the patient with pyelonephritis Does Dr. Sommers know of any studies, or has he done any studies, of the blood vessels in patients with contracted kidneys and unilateral or bilateral pyelonephritis?

DR SOMMERS. In our series of surgically treated cases of ostensible unilateral renal disease, we have not been fortunate in seeing a high rate of complete cure, and among nineteen cases available to the pathology department for study they fall into three approximately equal groups, with an excellent result, an intermediate result, and a poor result

In the studies made, the diagnostic feature of primary renal hypertension was a significant hyperplasia of the juxtaglomerular apparatus cells. This did not occur in the cases with intermediate or poor results. So I believe one can recognize a true surgical Goldblatt kidney by a juxtaglomerular cell hyperplasia which if significant will give a good prognosis

In the cases with poor results after nephrectomy there seems to be a piling up of granules, thought to be renin, both in the juxtaglomerular cells and also in tubular cells So I would say yes, there are two types of renal hypertension one primary, curable, the Goldblatt-type kidney, and the other described in pyelonephritis with hypertension, secondary renal hypertension, either unilateral or bilateral, which in our experience has not usually been curable by nephrectomy.

As regards the blood vessels in the kidneys, they were mapped, and the per cent value of the tissue mapped was lowest for the group with

detailed chart on the crucial patients, including a history of urinary tract infection and a history of known hypertension.

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DR. PERKOFF. In our patients and in other patients with the hereditary forms of renal disease associated with nerve deafness amino acid pattern has been consistently normal. I should like to ask Dr. Dent if he has

without a family history of hypertension there is almost a 40 per cent incidence of hypertension So it cannot leave any great doubt that there is a causal relationship betwen pyelonephritis and hypertension

Another point concerns the gravity of the disease. There is no doubt that the more gravely the renal function is affected, the higher the incidence of hypertension, but we have found an incidence of 30 per cent of hypertension even in those subjects with chronic pyelonephritis who had a normal glomerular filtration rate and only a very slightly affected tubular function

As for unilateral disease, my colleagues Dr. Prát and Dr Horrock have been studying the question, and they have found (and this is our only point of disagreement with Dr. Grieble) that unilateral disease, subdivided according to the same criteria, shows a higher incidence of hypertensive disease than can be found in cases of chronic cholecystitis.

reverse, hypertension without renal ischemia. That may seem academic, but I do think it is of some importance, because I believe that when we get deeper into the mechanisms involved we shall find it is not necessary to have ischemia but rather a change in the character of the pulse wave.

Also, I should like to add a word to what Dr. Kass said. I think his comments in general are true If you will recall a paper from the Mayo Clinic by Dr Braasch, there was no association between hypertension and either pyelonephritis or any kind of renal abnormality. At that time *this was accepted by those who had little use for renal types of hypertension* as a sort of finishing off of the whole idea.

Subsequently, little by little, people have chipped off various pieces of what was supposed to be a monolith, and so we now recognize that there are many patients, if they are properly selected, who have types of hypertension which are clearly renal, just as there are many patients with pyelonephritis who end up with hypertension. We see this all the time.

So I think there can be no doubt whatever that there is a direct association between pyelonephritis and hypertension. But if you turn it the other way, and say how many of these cases occur in the population, then certainly they are not a preponderance. I have always assumed, since I did the work on hypertension elicited by perinephritis, that the hypertension of pyelonephritis was due to the increase of interstitial tissue with increasing rigidity of the kidneys which in turn produced *a change in pulse characteristics*

Dr. Brod When we analyzed our series of 311 patients with chronic pyelonephritis diagnosed on clinical grounds, we took a control group, not just healthy subjects in a general population, but subjects affected by a chronic low-grade infection causing general symptoms similar to those of pyelonephritis, namely, an unselected group of 100 patients with chronic cholecystitis.

The over-all incidence of hypertension in chronic cholecystitis is 15 per cent. The over-all incidence of hypertension in chronic pyelonephritis is 60 per cent. If we divide the group with chronic cholecystitis and the chronic pyelonephritics into those over and under age 40, and subdivide these two groups into subjects with a positive family history [illegible] then in the subjects with [illegible] ily history [illegible] per cent

We cannot be sure that there is no coexistence of the two conditions, essential hypertension and renal disease, but if we take subjects under 40 years of age with a negative family history of hypertension, there is a zero incidence of hypertension. In subjects with chronic pyelonephritis

ents consistently identifiable in this fraction at pH 8.6 (Figure 1a). Modified Cohn Method 10 fractionation reveals at least two additional electrophoretic gradients.[8] Immunochemical examination of the electrophoretically separated normal urinary proteins has been reported only at pH 8.6.[8, 20] By this technique, albumin contains a single component present in both serum and urine. The urinary albumin exhibits a trailing component which may represent the material in gradient 4 (Figure 1b).

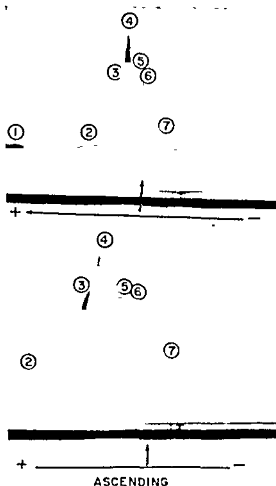
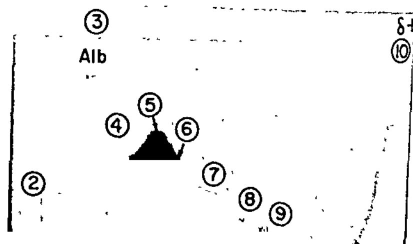
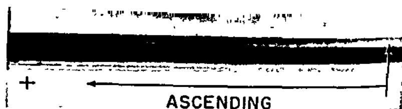


FIGURE 2 Ascending electrophoretic pattern of normal RS-1 urinary solids. Acetate buffer, pH 4.5, ionic strength 0.1, potential gradient of 6.6 v. cm., time 14,400 seconds (top photograph). Lower photograph is similar preparation after 11,600 seconds migration.

The RS-1 fraction contains all the serum proteins which appear in urine. Albumin is quantitatively the most abundant serum protein to appear in normal urine (averaging 15 mg. per 24 hours, range 6 to 32 mg. per 24 hours). Electrophoretically there are ten concentration gradi-





CONCENTRATION GRADIENTS APPEARING IN SERUM AND IN RS-1 FRACTION OF URINE TNDS AT pH 8.6

Moving boundary electrophoresis, Veronal buffer pH 8.6 0.1 M, electropotential 5.6 Volt per cm, time 14 400 sec

Descending toward Anode Negative Mobility – $\mu \times 10^{-5}$ cm^2/volt sec.

Normal Serum	Component			ALBUMIN		α-1		α-2	β	γ-1	γ-2	ε
	μ mean ± s.d.			6.64 ±0.14		3.45 ±0.17		4.34 ±0.24	3.22 ±0.22	2.42 ±0.24	1.55 ±0.23	
Normal Urine RS-1	Component	①	②	③	④		⑤	⑥	⑦	⑧	⑨	⑩
	μ mean ± s.d.	10.24 ±0.14	7.79 ±0.13	6.76 ±0.12	6.06 ±0.17		4.88 ±0.36	4.02 ±0.43	3.31 ±0.32	2.41 ±0.24	1.72 ±0.24	

FIGURE 1a. Ascending …

1b. Comparison of electrophoretic mobilities of gradients in normal serum and RS-1 solids of urine. Note that the "alpha globulins" of urinary RS-1 encompass three gradients, 4, 5, and 6.

although febrile episodes of 99 to 100° F. had been recorded by three the group. These patients were studied within 3 days after an acu attack had subsided under sulfonamide or Furadantin therapy. Drugs we discontinued during the collection period. The urinary sediment con tained no abnormal numbers of erythrocytes, leukocytes, epithelial cells tubular casts, or bacteria during the collection period. These patients were electively admitted to the Renal Diagnostic Ward for collection of specimens and subsequent diagnostic tests.

Group II. Recurrent Urinary Infection with Severe Bilateral Renal Disease

Five adult patients (3 female, 2 male), with recurrent exacerbations of chronic pyelonephritis over periods of 6 to 14 years, were selected for this group.

The precipitating factors in the initial attacks were obscure, but the nature of the infecting organism appeared to be an important cause for chronicity. The principal organisms were *Proteus mirabilis* and/or *Proteus rettgeri* in three patients, and *Aerobacter aerogenes* in two. Secondary infection with coliform bacilli or enterococci appeared to be the precipitating cause of the acute exacerbation of the disease in four of the group. Patients were admitted to the hospital for study during an acute episode of "pyelonephritis," with febrile response greater than 102° F. The uncentrifuged urine contained many leukocytes, erythrocytes, and, usually, renal tubular casts and bacteria. The admission BUN was slightly increased above normal in three of the group. The urine collections were begun as soon as the patient had been afebrile for 48 to 72 hours. During the study period the uncentrifuged urine contained only a few formed elements. The patients were continued on penicillin, chloramphenicol, or kanamycin.

Group III. Urinary Infection with "Healed" Renal Disease

Ten patients (4 female, 6 male) were included in this group who had had acute and severe pyelonephritis. The etiology was either "idiopathic" or initially associated with pregnancy, prostatitis, or instrumental examinations of the urinary tract. They had been on various regimens of antimicrobial therapy for continuous periods of 6 weeks to 14 months. None of these patients had any evidence of active infection at the time of study. Sedimentation rates, urinary cultures, and colony counts were all within the normal range of variation.

Five patients (2 female, 3 male) with evidence of residual impairment of renal function formed group III-A. These patients had maximal urea clearance values between 26 and 44 per cent of normal. The phenol red excretion was delayed in the first half hour, and the total was usually

The *alpha* globulins contain four components which are present in both serum and urine, two components which are present in serum but not in urine, and one component present in urine but not in serum. The *beta* globulins contain two components common to both urine and serum, but beta-lipoprotein and three other beta globulins present in serum have not been detected in normal urine. The *gamma* globulins of urine have a tendency to duplication of the precipitating bands, presumably due to alterations in molecular size of the urinary gamma globulin.[20] The electrophoretic mobility and antigen-antibody specificity of the urinary proteins are not reliable criteria for judging their molecular size. Several studies indicate that the normal urine contains few molecules of blood plasma origin larger than albumin and probably none of molecular weight greater than 100,000.[1, 3, 21] Moreover, the electrophoretic mobilities at pH 4.5 (Figure 2), relatively high glucide content, isoelectric points between pH 2.2 and 4.2, and low content of sulfhydryl groups of the normal RS-1 urinary solids are all inconsistent with the presence of the high-molecular-weight globulins typical of blood serum.[5, 11]

Total Nondialyzable Solids of Pyelonephritic Urine

An effort was made to confine the present study to alterations in urinary nondialyzable solids produced by bacterial invasion of the urinary system per se. Subjects were chosen from a group of adult patients who had been under observation for 26 months to 14 years. Eliminated from the study were all patients with hypertension, anatomic or physiologic abnormalities of the major conduits of the urinary system (other than minor cicatricial changes in the calyces), calculus formation, pregnancy, liver disease, any gross abnormality of serum proteins (either in quantity or electrophoretic pattern), any history of renal disease in childhood, any history of tuberculosis, and any azotemia persistent beyond an acute inflammatory exacerbation of the disease. The remaining patients were grouped, according to their history and available clinical information, into three groups.

Group 1. Recurrent Urinary Infection Without Clinical Evidence of Renal Disease

This group consisted of five married women, ages 23 to 32 years, who had never been pregnant and who had no history of urinary tract disease prior to marriage. Each had experienced recurrent symptoms of "cystourethritis" through periods of 3 or more years. Cultures and colony counts had revealed coliform organisms in the urine on more than one occasion. None of these patients had had symptoms of renal disease,

although febrile episodes of 99 to 100° F. had been recorded by three of the group. These patients were studied within 3 days after an acute attack had subsided under sulfonamide or Furadantin therapy. Drugs were discontinued during the collection period. The urinary sediment contained no abnormal numbers of erythrocytes, leukocytes, epithelial cells, tubular casts, or bacteria during the collection period. These patients were electively admitted to the Renal Diagnostic Ward for collection of specimens and subsequent diagnostic tests.

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Five adult patients (3 female, 2 male), with recurrent exacerbations of chronic pyelonephritis over periods of 6 to 14 years, were selected for this group.

The precipitating factors in the initial attacks were obscure, but the nature of the infecting organism appeared to be an important cause for chronicity. The principal organisms were *Proteus mirabilis* and/or *Proteus rettgeri* in three patients, and *Aerobacter aerogenes* in two. Secondary infection with coliform bacilli or enterococci appeared to be the precipitating cause of the acute exacerbation of the disease in four of the group. Patients were admitted to the hospital for study during an acute episode of "pyelonephritis," with febrile response greater than 102° F. The uncentrifuged urine contained many leukocytes, erythrocytes, and, usually, renal tubular casts and bacteria. The admission BUN was slightly increased above normal in three of the group. The urine collections were begun as soon as the patient had been afebrile for 48 to 72 hours. During the study period the uncentrifuged urine contained only a few formed elements. The patients were continued on penicillin, chloramphenicol, or kanamycin.

Group III. Urinary Infection with "Healed" Renal Disease

Ten patients (4 female, 6 male) were included in this group who had had acute and severe pyelonephritis. The etiology was either "idiopathic" or initially associated with pregnancy, prostatitis, or instrumental examinations of the urinary tract. They had been on various regimens of antimicrobial therapy for continuous periods of 6 weeks to 14 months. None of these patients had any evidence of active infection at the time of study. Sedimentation rates, urinary cultures, and colony counts were all within the normal range of variation.

Five patients (2 female, 3 male) with evidence of residual impairment of renal function formed group III-A. These patients had maximal urea clearance values between 26 and 44 per cent of normal. The phenol red excretion was delayed in the first half hour, and the total was usually

reduced below the normal range. Maximal specific gravity ranged between 1.014 and 1.020 The renal biopsies indicated some cicatricial changes varying from membrane thickening to glomerulosclerosis in those specimens where any abnormality was detectable.

Patients in group III-B (2 *female*, 3 *male*) *exhibited only equivocal or* no evidence of impaired renal function. With respect to the duration, frequency, or severity of prior attacks of urinary infection, they were indistinguishable from group III-A.

Three or more 24-hour urine specimens from each of the above 20 subjects were analyzed by the previously described methods[4,6,9,11] No diagnostic procedures, other than those which would be accomplished by collections of blood and urine samples, were permitted during the week prior to completion of the collection period.

Results

The quantitative variations in the three major fractions of total nondialyzable urinary solids are presented in Table I. Experiments have shown the recovery of UF-O to be essentially complete. The physical

TABLE I Comparison of Weights[a] of Primary Fractions of Nondialyzable Urinary Solids for Normal and Pyelonephritic Subjects

SUBJECTS (Number)[b]	R-1			RS-1			UF-O		
	MEAN	RANGE	± sd	MEAN	RANGE	± sd	MEAN	RANGE	± sd
NORMAL (12)	90	35-168	39	59	22-124	25	265	146-522	100
PYELONEPHRITIS									
GROUP I (5)	60	28-83	24	51	44-63	9	308	198-373	77
GROUP II (5)	248	132-464	125	550	223-744	234	607	281-988	280
GROUP III									
(5) A	101	35-175	61	487	222-657	203	494	134-927	294
(5) B	71	56-97	14	55	11-101	33	394	144-668	189

[a] Mg per 24 hrs
[b] 1 to 8 determinations per subject

character of the nonultrafiltrable material and the necessity for using several filter membranes for a single 24-hour collection of pathologic urine have caused the mechanical losses of R-1 and RS-1 to be higher than for normal urine. In addition, the total solids contain slightly more "bound" water than can be found in the fractionated solids The sum of the weights of UF-O, R-1, and RS-1 range generally from 60 to 70 per cent of the actual total nondialyzable solids (as determined on a

separate aliquot). The values for R-1 and RS-1 in Table I must therefore be regarded as minimal. The ratio R-1/RS-1 should be reasonably correct in each case, however.

The ultrafiltrable solids (UF-O) of urine are increased in patients with established pyelonephritis. This increase is not one of the earliest changes observed, since it is not detectable in group I patients, but it is one of the most persistent abnormalities, being significantly present in apparently healed patients, group III-B (Table I) The UF-O increase is rarely more than four times the normal mean, as compared with values up to fifteen times the normal for RS-1. Present lack of knowledge as to the composition or origin of the UF-O solids in health precludes further consideration of their significance in disease

The insoluble, nondialyzable R-1 solids are significantly elevated only in acute infections, group II. Some contribution to the weight of this fraction is undoubtedly made by renal tubular casts, lysed cells, and possibly bacteria. However, if these solids are primarily a result of epithelial secretion, one may surmise that an increase in quantity reflects an epithelial response to bacterial stimulation Similarly, one may interpret the lower values for R-1 in groups I (minimal disease) and III-B (healed) as indicative of a healing or regenerating epithelium, which has not acquired the mucoprotein-rich surface layers of aging, normal transitional epithelium.

The RS-1 fraction, presumably representative of serum substances in urine, received the primary emphasis in the present study (Table II, Figures 1 and 2). Centrifugation at a higher speed (20,000 × g) than that used for normal samples (1000 × g) was found to remove a persistent trace of suspended R-1 material The light-scattering properties of these mucoids may effect a veil-like interference with the schlieren patterns extending from the initial boundary to the alpha globulin sectors of the cell.

Group I. No Evident Renal Disease

This group exhibits no significant abnormality in excretion rate of RS-1 The schlieren diagrams at pH 8 6 are not greatly different from variations in the normal (Table II) If one compares the electrophoretic patterns at pH 4 5 of normal RS-1 with Figure 3, the qualitative difference is immediately apparent. Substances corresponding in electrophoretic mobility to the serum globulins B and C, which are not seen in normal urine, are here present in detectable quantities (Table III)

Group II. Severe Renal Disease

The RS-1 excretion is markedly increased, with considerable variation among individuals (Tables I and II). The percentage distribution, or

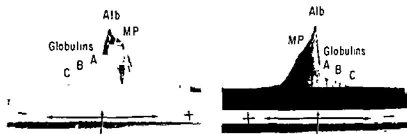


FIGURE 3 Electrophoretic patterns of RS-1 solids of patient from group I (no evident renal disease). Concentration of solids 16 mg/ml. Conditions as for Figure 2, time 14,400 seconds

relative concentration, of the various gradients of the electrophoretic patterns is much more constant than the total excretion rates of RS-1. The variations from normal, which are barely perceptible in group I, are here intensified. The relative concentrations of the RS-1 "alpha globulins" are reduced (Table II). The reduction in relative concentration of RS-1 material having the mobility of alpha globulins may reflect a physical change in the soluble uromucoid [8, 18] so that, although the total

TABLE II. AREA CONTRIBUTION AND EXCRETION RATES OF EACH ELECTROPHORETIC GRADIENT (pH 8.6) FOR NORMAL AND PYELONEPHRITIC SUBJECTS*

SUBJECTS (NUMBER)[b]		RS-1 TOTAL mg/24 hrs[c]	ELECTROPHORETIC CONCENTRATION GRADIENTS[a] 2 (Ic-2)		3 (Alb)		4 (α-1)		5+6 (α-2)		7 (β)		8 (γ-1)		9 (γ-2)	
			%[d]	mg[e]	%	mg	%	mg	%	mg	%	mg	%	mg	%	mg
NORMAL (12)	MEAN	59	2.6	1.6	25.9	15.2	4.6	2.7	45.0	26.4	10.1	6.0	6.0	3.5	5.7	3.4
	RANGE	22–104	1.3–4.6	0.6–3.5	14.5–35.9	5.7–32.2	2.5–8.4	1–6	35–63	10–36	6.2–10.3	2–13	3–10	1.3–7.6	3.3–9.6	1.3–7
	± sd	(23)	(1.2)		(6.3)		(1.8)		(9.1)		(2.7)		(1.9)		(2.0)	
PYELONEPHRITIS																
GROUP I (5)	MEAN	51	1.3	0.6	23.6	10.9	7.4	3.4	31.0	14.1	19.1	8.7	11.0	5.6	6.4	3.0
	± sd	(9)	(0.2)	(0.1)	(6.5)	(2.4)	(1.7)	(0.9)	(11)	(4.8)	(3.9)	(2.3)	(4.3)	(3.2)	(3.0)	(3.5)
GROUP II (5)	MEAN	550	1.4	8.3	21.9	124.0	2.8	16.0	24.0	130.0	7.9	42.0	26.0	132.0	17.0	100.0
	± sd	(234)	(0.4)	(3.3)	(4.7)	(65)	(1.1)	(11)	(7)	(78)	(2.3)	(20)	(9)	(58)	(8)	(69)
GROUP III A (5)	MEAN	487	—	—	71.0	345	2.0	10.0	8.0	39.0	7.5	37.0	7.4	36.0	4.3	21.0
	± sd	(183)			(8)	(160)	(1)	(6)	(3)	(29)	(5)	(9)	(7)	(19)	(1.5)	(7)

[a] Numbered in accord with Fig 1
[b] 3 to 8 determinations per subject
[c] Lyophilized dry weight (see text)
[d] Per cent of total area (by planimetry of traced photograph magnification X 3) exclusive of gradient 10
[e] Calculated from mean values obtained from ascending and descending patterns of each sample

* Expressed as per cent of total and as milligrams per 24-hour excretion

TABLE III. CONTRIBUTION OF EACH ELECTROPHORETIC GRADIENT TO THE TOTAL PATTERNS FOR SERUM AND RS-1 FRACTION*

SUBJECTS (Number)[c]	URINE RS-1[a] pH 4.5					SERUM[a] pH 4.5					SERUM[b] pH 8.6					
	MP	Alb	GLOBULINS A	B	C	MP	Alb	GLOBULINS A	B	C	Alb	α 1	α 2	β	γ 1	γ 2
NORMAL (5)	66	29	5	0	0	35	54	115	10	21	555	64	72	121	51	110
PYELONEPHRITIS GROUP I (5)	493	349	68	58	32	-	-	-	-	-	-	-	-	-	-	-
GROUP II (5)	62	484	149	133	172	68	501	41	70	32	452	63	124	138	29	193
GROUP III A (5)	62	760	67	59	52	71	586	138	117	88	482	62	160	145	38	113
GROUP III B (5)	238	626	108	18	10	56	514	107	53	27	521	73	141	137	34	94
ADULT NEPHROSIS (1)	0	628	136	146	90	68	213	120	580	19	167	84	190	392	35	113

[a] Acetate buffer, ascending boundary only
[b] Veronal buffer, mean values for ascending and descending boundaries
MP - all gradients of net negative mobility greater than albumin
Globulins gradients of net positive mobility lettered in order of increasing mobility
[c] Mean values for 1 to 3 determinations per subject

* Expressed as per cent of total planimeter units

in urine is increased, a relatively greater proportion of it appears as the insoluble R-1 rather than the soluble "alpha globulins" It is notable that, whereas the component with the precise mobility of serum alpha-2 is obscured in normal and group I urinary RS-1, it appears as the major component of the alpha globulins in group II (Figures 4 and 5) Components with the mobility of alpha-2 globulins are normal or slightly elevated in serum of these patients (Table III, Figure 5). Areawise, the second largest component of the alpha globulins of group II has a mobility approximating that of component 6 of normal urine (Figures 1 and 5). Thus, the alpha components (4, 5, and 6, Figure 1) of urine have distinct differences, both in their solubility properties[10] and in their concentrations in pyelonephritic urine

The mobility at pH 8.6 of the beta-1 globulins is the same in serum, in pyelonephritic urine, and in nephrotic urine (Figure 6)

The most striking deviation from normal in these group II subjects is an absolute and relative increase in material having at both pH 8.6 and 4.5 the mobility of true gamma globulins (Figures 4 and 5) These patients have a significant increase in serum gamma globulins (Table III).

The relative quantities of RS-1 mucosubstances (MP)* are quite different from the normal in all the patients, but the absolute excretion in milligrams per 24 hours is surprisingly constant (Table IV) In groups I and III-A the total amounts of hexose and hexosamine per 24 hours

* MP refers to the sum of electrophoretic components, other than albumin, with net negative charge (mobility toward anode) at pH 4.5

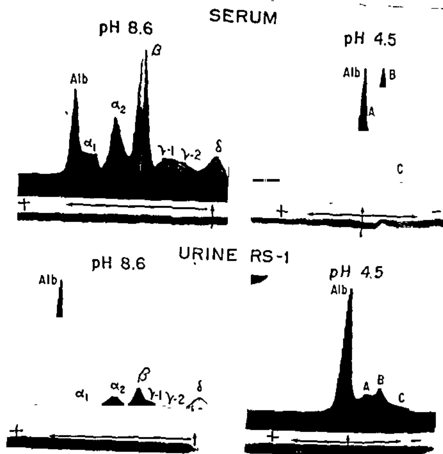


FIGURE 6 Electrophoretic patterns of serum and of urinary RS-1 solids from adult patient with classical nephrotic syndrome. Ascending patterns Conditions of electrophoresis at pH 8 6 as for Figure 1. Conditions of electrophoresis at pH 4.5, acetate buffer ionic strength 0 1, gradient 5.2 v /cm, time 14,400 seconds.

TABLE IV. ESTIMATE OF MUCOSUBSTANCES IN NORMAL SERUM AND IN NORMAL AND PYELONEPHRITIC RS-1 FRACTIONS*

SUBJECTS	Electrophoresis, pH 4 5 % of area[a]			Composition % of lyophilized weight				Excretion milligrams per 24 hr		
	MP	Alb	Glob	Hexose (Anthrone)	Hexosamine HCl	Nitrogen	Protein (N × 6 25)	MP*	Hexose	Hexosamine HCl
NORMAL SERUM[b]	3 5	54	42 5	<1 0	1 5	14 0	87 5	—	—	—
RS-1	66	29	<5	11 9	6 1	9 4	60 6	39	7	3 6
PYELONEPHRITIS										
GROUP I	49 3	34 9	15 8	9 3	3 1	12 2	76 3	25	5	1 6
GROUP II	6 2	48 4	45 4	5 9	3 8	14 0	87 5	34	32	21
GROUP III-A	6 2	76 0	17 8	4 2	3 3	15 5	96 8	30	20	16

[a] Per cent of total area from traced photograph

[b] Dialyzed and lyophilized as urinary TNDS

* Estimate of excretion from combination of electrophoresis and analysis.

increased hexose and hexosamine are evidently present as an electrophoretically nondiscernible heterogeneous or adsorbed substance or one which is immobile and obscured by the albumin gradient at pH 4.5 or by the delta boundary at pH 8.6. The origin of these substances may be either bacterial polysaccharides or inflammatory tissue exudates, as well as the many noninfectious intrinsic sources of mucosubstances.

Group III-A. Healed with Renal Impairment

These patients' urine is characterized by a persistently high quantity of RS-1 with both a relative and an absolute increase in albumin. The considerable range of variation in the gamma-1 globulin is reflected in the standard deviation for these values (Table II). On the whole, the

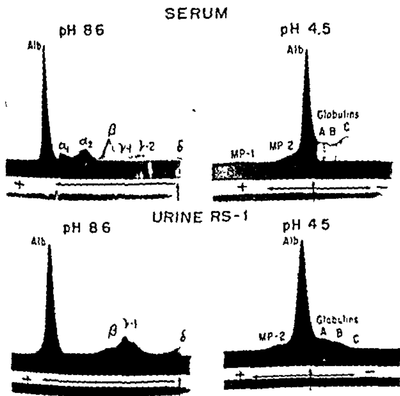


FIGURE 7. Electrophoretic patterns of serum and of RS-1 solids from a typical group III-A patient. Conditions of electrophoresis as for Figure 6. Concentration of urinary solids 16 mg/ml

These substances remain in the urine of damaged kidneys after bacteria are absent, but disappear as renal function approaches the normal.

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*Renal Mechanisms and Hematopoiesis**

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It is now generally accepted that the dynamic equilibrium of erythropoiesis is regulated by a substance commonly referred to as erythropoietin. A number of reviews[1, 2, 7, 29-32] trace the development of the concept of humoral control of erythropoiesis, which originated with Carnot in 1906.[8] The rapid progress in this field of research in the past decade stems especially from the work of two groups of investigators, namely, Reissmann,[26] who may be credited with providing acceptable proof of the existence of humoral control of erythropoiesis, and Borsook,[3] who demonstrated that this humoral factor could be chemically extracted and partially purified from the plasma of anemic animals.

The efforts of our laboratory have been concerned with (1) the search for a sensitive bioassay of erythropoietic activity, (2) the study of the physiologic parameters which control production of the hormone, (3) the attempts to determine the site of formation of the hormone, and, finally (4) the production and purification of a sufficient amount of the hormone to provide material for chemical characterization and clinical trials. These studies have recently been reviewed.[12, 13, 17] The present communication contains no essentially new data and will deal largely with those of our studies that have pointed to the kidney as the (possible) major organ of erythropoietin production.

Assay Methods

As previously reported,[17] our assay methods consist of rather simple maneuvers which produce a relative or absolute polycythemia in the recipient animal, and thus reduce the rate of erythropoiesis nearly to zero. The erythropoietic response of these animals to the parenteral administration of normal plasma is minimal, whereas the response to plasma rich in erythropoietin or to active fractions is regularly many times above control values.

*From Argonne Cancer Research Hospital, operated by the University of Chicago for the United States Atomic Energy Commission.

reduces, but does not eliminate, erythropoietin production, even though the uremia (BUN elevation) is comparable in both experimental conditions (Table II).

TABLE II. THE EFFECT OF PLASMA OBTAINED FROM RATS THAT HAD BEEN SUBJECTED TO NEPHRECTOMY OR LIGATION OF THE URETERS FOLLOWED BY Co^{++} STIMULATION, UPON THE INCORPORATION OF Fe^{59} INTO THE RED BLOOD CELLS OF STARVED RECIPIENTS

Surgical Procedures*	Stimulus	Time of Removal of Blood from Donor After Co^{++} Injection (hours)	BUN of Plasma†	Assay of Donor Plasma in Recipient Starved Rats Using Percentage Fe^{59} Incorporation Response of Recipient to Plasma Preparation
None	None	—	—	3 3 (0 2)‡
None	Cobalt (167 μM/Kg)	12	22	6 6 (1 1)
Nephrectomy	Cobalt	12	99	3 3 (0 5)
Ligation of the ureters	Cobalt	12	95	9 8 (0 1)

* Immediately following surgery, the rats (Sprague-Dawley, 350 Gm) were injected subcutaneously with cobaltous chloride, and 12 hours later the blood was collected via cardiac puncture The plasma thus obtained was assayed in starved rats by standard procedures

† Blood urea nitrogen

‡ Standard error

The effect of nephrectomy on the rate of disappearance of erythropoietin from the plasma (Figure 1) also has been studied as part of this series of experiments Animals were given injections of cobaltous ion and, at intervals thereafter, nephrectomy was performed and observations made to determine the rate of fall-off of erythropoietin titer. It is evident that bilateral nephrectomy at 4 hours after the injection of cobalt prevents any rise of titer, while nephrectomy at 8 hours causes a rapid cessation of erythropoietin production The fact that the fall-off curves are parallel indicates that the loss of activity from the plasma is not significantly different from that seen in the normal animals that have been given a single dose of cobalt. Unilateral nephrectomy does not interfere with the normal production of erythropoietin in these rats. These data would seem to lend support to the idea that the hormone is elaborated solely (within limits of detection by the starved rat assay) by the kidney after cobalt stimulation.

In yet another experiment, rabbits were bled by a standard method (hematocrit about 25) and then subjected to bilateral nephrectomy or related procedures Twelve hours after surgery the animals were exsanguinated. The chilled plasma was brought to 5 per cent final concentration of perchloric acid. The supernatant solution was dialyzed free of acid,

concentrated, and assayed for erythropoietin in polycythemic mice. As shown in Figure 2, bilateral nephrectomy reduces the erythropoietin titer to the normal range, whereas the titer of animals subjected to bilateral ureter ligation is comparable to those of unoperated anemic animals.

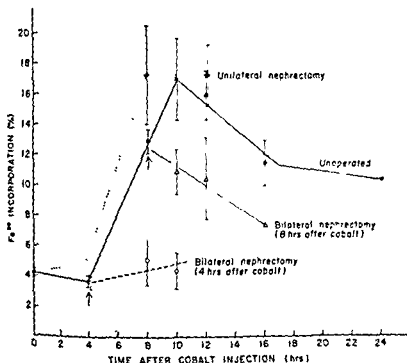


FIGURE 1. Effect of plasma obtained from rats that had been subjected to nephrectomy and cobalt stimulation on the incorporation of Fe^{59} into the red blood cells of starved recipients. Sprague-Dawley rats, 310 Gm., were used as donors. These were injected with 75 micromoles of $CoCl_2$ at zero time, nephrectomy [illegible] as indicated by arrows, and exsanguina- [illegible] *Kinetics of Cellular Proliferation*. New York: Grune and Stratton, 1959, p. 349.[12]

A report[16] that nephrectomized rats responded to hypoxic hypoxia by the production of erythropoietin caused us to reinvestigate this matter. Although it seemed highly improbable, the possibility existed that response to the low oxygen stimulus was qualitatively different from that induced by hemorrhage.

Urinary Excretion of Erythropoietin

Erythropoietin has been demonstrated in the urine of animals after phenylhydrazine stimulation.[11] It has also been demonstrated in the urine of patients with certain disease states[9, 25, 30] A rapid fall in the plasma and urinary titers of erythropoietin occurs following transfusion.[10, 11] Erythropoietin has not been demonstrated in the urine of normal animals or man, but this may only indicate the inadequacy of our present assay methods. This problem should be explored thoroughly, since such studies might reveal whether erythropoietin is the threshold substance it appears to be.

We have demonstrated conclusively in one patient a fivefold relationship between urinary and plasma activity (Table IV).

TABLE IV. Plasma and Urinary Erythropoietin Titer, Specimens from Patient with Aplastic Anemia. Assay in Starved Rat

Plasma		Urine	
Volume*	Assay Result† (Per Cent)	Volume*	Assay Result† (Per Cent)
4 ml.	36 0	20 ml.	37 0
2 ml	31.0	10 ml	33 3
1 ml.	25 6	5 ml.	25 7
0 5 ml.	17.8	2.5 ml	15 2
0	4 5	0	3.7

* All specimens concentrated or diluted to volume of 4 ml, given as 2 daily injections of 2 ml each

† Incorporation of a tracer dose of Fe^{59} into peripheral red cells

While the resolution of differences in both results and interpretations from the various laboratories awaits further, more decisive experimentation, some indirect evidence concerning the role of the kidney in the elaboration of erythropoietin is worthy of attention. Naets[21, 22] has shown that nephrectomized dogs, kept alive by peritoneal dialysis, have a severely suppressed rate of erythropoiesis, while ureter-ligated dogs undergo only a minimal depression of this function.

Recently, Osnes[23] has published some data indicating that in mice, x-irradiation of the kidneys with large doses can cause a severe uremia along with anemia: in fact, the anemia is apparent before the uremia becomes severe. If part of the kidney is shielded from x-irradiation, uremia still results, but anemia does not occur.

The clinical observation by Richet[27] in which he describes cessation of erythropoiesis in acute renal shutdown is of considerable interest as

recovery from the renal crisis proceeds, resumption of erythropoiesis is observed.

Gurney and associates[10] have demonstrated that 7 out of 7 anemia patients with uremia did not have elevated erythropoietin titers, even though they were as anemic as other patients who did have increased amounts of erythropoietin in their plasma, and similar results were recently reported in 15 of 16 instances where plasma from anemic uremic patients was assayed.[7]

Finally, it should be mentioned that polycythemia has been observed in association with unilateral and bilateral renal disease. Forssell[4] reviewed the literature relative to this clinical observation, and reports a substantial number of cases in which the polycythemic state was corrected following surgical removal of a diseased kidney.

In spite of our laboratory evidence and that of others pointing to the kidney as the major, if not the sole, producer of erythropoietin, we are continuing the search for evidence which may be more direct than that already discussed. As further work in this field progresses, quantitative measurement of plasma erythropoietic activity should be possible. We should then be in a better position to determine whether the kidney is the sole producer of erythropoietin(s) or a co-producer.

Summary

By use of short-term bioassay methods we have established that the kidney is the major, if not the sole, site of production of the hormone erythropoietin.

Nephrectomized animals fail to respond to stresses such as anemic anoxia, hypoxic anoxia, and cobalt, while animals with comparable degrees of uremia, induced by ureter ligation, respond about as well as unoperated, stressed animals.

The possible role of erythropoietin lack in the anemias of renal disease is discussed briefly.

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*Prolonged Potassium Deficiency and Chronic Pyelonephritis in Man and Animals**

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The multiple functional and structural renal changes produced in animals and man by potassium depletion (Table I) are now well recognized.[3, 5, 8, 9, 10, 11, 14, 16, 17, 18, 22, 26, 27, 30, 32, 35] In acute deficiency these

TABLE I. Renal Effects of Potassium Depletion *

Glomerular Changes
- (1) Lowered glomerular filtration rate and urea clearance[29, 32]

Functional Changes in Renal Tubules
- (1) Hyposthenuria or isosthenuria, vasopressin resistant[4, 8, 19, 30, 32]
- (2) Impaired para-aminohippurate extraction and excretion[29, 32]
- (3) Inability to establish minimal urinary pH, relative increase in urinary ammonia (NH_3)[5, 19]
- (4) Decreased urinary citric and other organic acids[19]
- (5) Impaired capacity to conserve sodium with low dietary sodium[19, 34]
- (6) Renal phosphaturia[19]

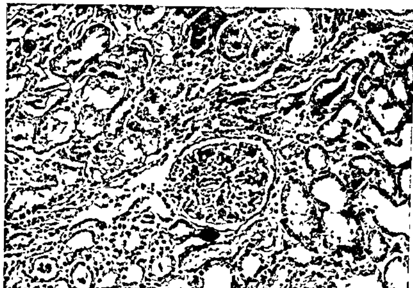
Structural Changes in Renal Tubules
- (1) Vacuolar (hydropic) nephropathy of proximal tubules[11]
- (2) Granular degeneration and atrophy of cells in distal and collecting tubules[17, 22, 33]
- (3) Increased intercalate cells in collecting tubules[26]

Histochemical Changes in Renal Tubules
- (1) Increased esterase in proximal tubules[27]
- (2) Decreased DPN diaphorase and increased TPN diaphorase in medulla and papilla[27]

Biochemical Changes in Renal Tubules
- (1) Decreased capacity to accumulate para-aminohippurate[16]
- (2) Intracellular acidosis[1]
- (3) Increased glutaminase D amino oxidase and carbonic anhydrase activity[11]

* Modified from Kleeman and Maxwell.[16]

* From the Department of Medicine, Presbyterian-St. Luke's Hospital, Research and Educational Hospitals and the University of Illinois College of Medicine, Chicago, Illinois. Supported by a grant from the United States Public Health Service (H 3038).



ognomonic tubular vacuole changes of potassium deficiency—is not present.

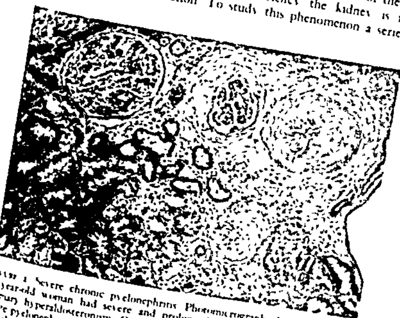


FIGURE 1b (Jan. 1958) Chronic pyelonephritis. Photomicrograph H & E ×145. A second renal biopsy was taken 40 months later. In the interval between renal biopsies she had intermittent diarrhea and hypokalemia.

TABLE II. RENAL BIOPSY STUDIES OF 18 PATIENTS WITH PROLONGED POTASSIUM DEFICIENCY

Chronic pyelonephritis	12
Interstitial fibrosis	5
Hydronephrosis	1
Hydropic degeneration	0

changes are usually rapidly reversed by feeding potassium[22] but in patients ill with chronic potassium deficiency, due to a variety of causes, histologic evidence of chronic pyelonephritis is frequently found by biopsy or at autopsy (Table II). This permanent and irreversible kidney lesion has been observed in patients who suffered prolonged potassium deficiency due to chronic diarrhea (Figures 1a and 1b),[23] prolonged vomiting,[14] severe primary hyperaldosteronism (Figure 2),[8,...] the Lignac-Fanconi syndrome,[7,20] ureterosigmoidostomy,[15] and in potassium deficiency of unknown origin.[15] It is possible that as a result of the prolonged metabolic stress of potassium deficiency the kidney is more susceptible to bacterial infection. To study this phenomenon a series of



...URE 2. Severe chronic pyelonephritis. Photomicrograph. H & E. ×90. A ...year-old woman had severe and prolonged potassium deficiency due to ...ary hyperaldosteronism. Over a 12-year illness she had three attacks of ...e pyelonephritis. She was found deficient of over 1000 mEq of potassium. ...al biopsy disclosed severe chronic pyelonephritis. Note the absence of hydropic degeneration of the tubule cells.



[illegible]

disclosed interstitial fibrosis. Note that hydropic degeneration — so-called pathognomonic tubular vacuole changes of potassium deficiency — is not present.



FIGURE 1b. (Jan. 1958) Chronic pyelonephritis Photomicrograph H & E ×145 A second renal biopsy was taken 40 months later. In the interval between renal biopsies she had intermittent diarrhea and hypokalemia.

potassium depletion on the kidney was studied by sacrificing experimental and control animals at various intervals of potassium depletion. Kidneys of six control and six potassium-deficient rats were studied with the electron microscope. These electron microscopic findings were correlated with ultramicrochemical studies of the nephron of control and potassium-depleted rats using Lowry's technique as modified by Bonting *et al.*[3]

Effects of prolonged potassium depletion on the kidney. Previous studies have shown[22, 23] that the rats made potassium-deficient on this diet died in 5 or 6 weeks. Therefore, rats were fed the low potassium diet for only 4 weeks and then slowly repleted for 3 weeks with potassium. This was accomplished by feeding them the same diet without the resin and giving them drinking water containing 0.1 per cent potassium chloride. This method of depleting and repleting the animals over a period of 7 weeks was repeated cyclically for periods of from 12 to 15 months.

For each experimental rat a male or female control animal was studied. These were fed the same diets as the experimental animals, but resin was not added to the food and 0.1 per cent potassium chloride was added to the drinking water in substitution for sodium chloride or sodium bicarbonate. At present, studies using weighed paired feedings are under way but no data are available.

Effect of experimental infection on the potassium-depleted kidney. In this study 160 rats were used. Eighty were experimental animals and eighty were well-fed controls. On the twenty-first day of potassium depletion 80 animals were divided into three groups. Forty were anesthetized and injected into the tail vein with 9×10^{8} freshly cultured viable *Escherichia coli* organisms* (group A). A second group of 20 potassium-deficient animals were anesthetized and injected intravenously with 9×10^{8} *E. coli* organisms and thereafter the right kidney was gently and firmly massaged for exactly 5 minutes (group B).[4] A third group of 20 animals was anesthetized with ether and received an injection of culture broth into the caudal vein (group C). The well-fed control animals were also divided into three paired groups (A_{con}, B_{con} and C_{con}) which were treated exactly as the experimental animals. After the rats were injected the potassium-deficient rats were maintained on the potassium-depletion regimen for a further 7 days. Then all rats were housed in colony cages of four animals and fed Purina fox chow *ad libitum*.

Some animals died (Table IV) but the remainder were sacrificed 6 weeks after injection of *E. coli*. Experimental and control animals were

*Standard strain fresh cultured viable *Escherichia coli* (strain O22 K negative) was isolated from the urine of a patient with pyelonephritis and supplied by Dr. G. G. Jackson, Department of Preventive Medicine, University of Illinois College of Medicine, Chicago.

experiments were designed which challenged the kidney of potassium-deficient animals to infection.

General Studies

The studies were divided into three parts: I. The effects of short-term potassium depletion on the kidney. II. The effects of prolonged potassium depletion on the kidney. III. The effect of experimental infection on the potassium-depleted kidney.

In all, 672 Sprague-Dawley rats were studied. Half were made potassium-deficient by feeding them a low potassium diet containing cation exchange resins with protein, vitamins, and mineral supplement (Table III). They were usually given tap water to drink, containing 0.45 per cent

TABLE III. Composition of Potassium-deficient Diet

Casein (U S Technical)	13%
Dextrin (Yellow Technical)	63%
Peanut oil	16%
Sodium polystyrene sulfonate resin	4%
Mineral mixture	4%
	100%
Multiple vitamins (Eli Lilly and Company)	3 ml
Mineral mixture	
$Ca_3(PO_4)_2$	20 Gm.
Na_2HPO_4 2 aq	86 Gm
$MgCO_3$	2 0 Gm
Na citrate	1 0 Gm.
Multiple vitamins (3 ml. contains)	
Thiamin hydrochloride	5 mg
Riboflavin	5 mg
Pyridoxine hydrochloride	5 mg
Pantothenic acid (or Sodium pantothenate, racemic)	15 mg
Nicotinamide	375 mg
Vitamin B_{12}, crystalline	15 mcg
Vitamin A, synthetic	25,000 units
Vitamin D, synthetic	5,000 units

sodium chloride. In some experiments the rats drank either tap water or tap water containing 0.9 per cent sodium chloride.

Morphologic studies were made of hematoxylin and eosin stained serial sections of all control and potassium-depleted rat kidneys.

Effects of short-term potassium depletion. The effect of short-term

anesthetized with ether. The abdomen was shaved and sterilized with 2 per cent iodine. Using complete aseptic operative techniques, the abdomen was opened. Blood was drawn from the inferior vena cava from all animals for bacteriologic and biochemical studies (nonprotein nitrogen, serum protein electrophoresis, and serum potassium). The urinary bladder and spleen were removed and each was ground with sterile sand and suspended in sterile water. Serial dilutions (10^2, 10^4, 10^6) were made of the blood and tissue suspension and the individual material was cultured on blood agar. The kidneys, pelvis, and ureters were divided into two halves by slicing transversely through the papillae. One half kidney was placed in 10 per cent neutral formalin for serial histologic examinations. The other half was placed in a tube of trypticase phosphate broth and



Figure 4a. Control rat. Note the smooth fine coat of hair.

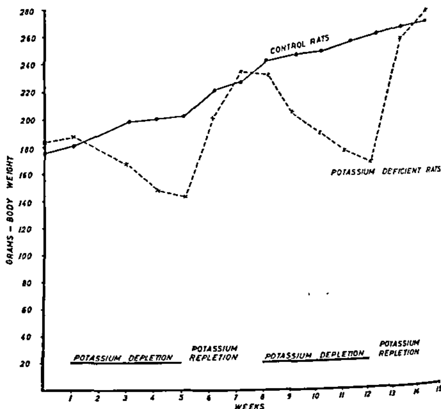


Figure 4b. Potassium-deficient rat. This photograph was taken 21 days after a potassium-depletion diet was started. This rat had paralysis of the hind legs. Note the rough coat of hair.

macerated with a sterile pestle. Four hours later serial dilutions (10^2, 10^4, 10^6, and 10^8) were made and the undiluted material and diluted material were incubated on eosin-methylene blue plates for 24 hours. Colony counts and bacteriologic identification were carried out.

TABLE IV. Mortality Following Intravenous Injection of *Escherichia coli*

	Potassium-depleted Rats			Control Rats		
	Injected with *E coli*		Injected with Broth	Injected with *E coli*		Injected with Broth
	No Massage A	Massaged B	C	No Massage A_{con}	Massaged B_{con}	C_{con}
Number of rats	40	20	20	40	20	20
Died within 1 day of injection	4	5	0	0	0	0
Died between 1 and 6 weeks	7	4	2	8	3	0
Sacrificed	29	11	18	32	17	20



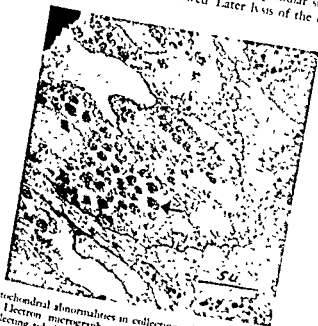
[illegible] on the body weight of experimental [illegible] over two periods [illegible] eight gain in the drinking water.

throughout the cytoplasm of every collecting tubular cell. Moreover, these granules were also noted within the capillary endothelial cells of the papilla tip. A prominent basophilic stroma separated the granule-laden cells. These morphologic changes were rapidly reversed to normal when the rats were repleted with potassium.

Electron Microscopic Studies

Electron microscopic abnormalities were limited to the collecting tubules of the distal papillae. Structures believed to be degenerated mitochondria were noted (Figures 6a and 6b) in various stages of degeneration. They are believed to be mitochondria because first, no normal mitochondria were found, second, a double membrane similar to the mitochondrial wall was noted surrounding the degenerated structure and third, cristae could be seen within the matrix of the degenerated structure.

The earliest changes appeared to be a swelling of the mitochondria and development of a granular matrix. Formation of globular structures with variable osmiophilic properties followed. Later lysis of the double-walled



6a Mitochondrial abnormalities in collecting tubules of papillae of potassium ...t rat. Electron micrograph ×1100. Mitochondrial abnormalities are seen ...he collecting tubule cells in the distal papillae of the potassium depleted rat ...mitochondria are not seen. Arrow points to abnormal mitochondria contain... ...e. Note mitochondrial abnormalities seen within the endothelial cells of the capillaries.

Results

After 21 days, the rats on a potassium-depleted regimen became lethargic and lost weight (Figure 3), their coats became shaggy (Figures 4a and 4b), and in some the hind feet were paralyzed. On repletion, the potassium-deficient rats rapidly became alert, the paralysis disappeared, and their coats returned to normal. Within 7 to 10 days on potassium depletion their weight equaled that of the paired control rats. After prolonged periods of potassium depletion and repletion, the experimental animals always regained weight to equal the paired controls. Serum protein electrophoretic studies of both groups were similar

I. Effects of Short-term Potassium Depletion on the Kidney

Gross and Microscopic Findings

There was an increase in the size of the kidneys of experimental animals This was due to enlargement of the outer medullary zone and papillae. The most striking histologic lesion noted was the accumulation of numerous eosinophilic oval-shaped granules of various sizes in the collecting tubules of the distal papillae (Figure 5). These granules were identical with those described by Spargo [35] and they were scattered



Figure 5. Eosinophilic granules in collecting tubules in papilla apex. Photomicrograph. H & E ×840. Kidney section taken from the papilla tip of a potassium-depleted rat. Numerous eosinophilic granules are noted in every cell of the collecting tubule including the endothelial cells of the capillaries

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FIGURE 6a Mitochondrial abnormalities in collecting tubules of papillae of potassium-deficient rat. Electron micrograph ×3300. Mitochondrial abnormalities are seen within the collecting tubule cells in the distal papillae of the potassium-depleted rat Normal mitochondria are not seen Arrow points to abnormal mitochondria containing cristae Note mitochondrial abnormalities seen within the endothelial cells of the capillaries

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FIGURE 5. Eosinophilic granules in collecting tubules in papilla apex. Photomicrograph: H & E ×840. Kidney section taken from the papilla tip of a potassium-depleted rat. Numerous eosinophilic granules are noted in every cell of the collecting tubule including the endothelial cells of the capillaries.

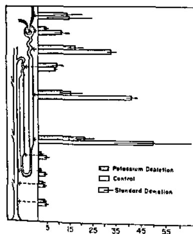


FIGURE 7a Alkaline phosphatase activity (moles of substrate turned over at 37° C per kilogram dry weight per hour) of control and potassium-deficient rats Schematic representation of the nephron is on the left The value of alkaline phosphatase activity is on the right. The values for control rats are open bars, for potassium-depleted rats shaded bars The line at the end of each bar represents the standard deviation

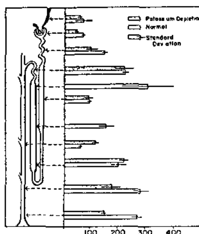


FIGURE 7b Lactic dehydrogenase activity (moles of substrate turned over at 37° C per kilogram dry weight per hour) of control and potassium-deficient rats This is a schematic representation of the nephron on the left and the values for the animals on the right The control animals have clear bars and the potassium depleted rats shaded bars Note the marked decrease in lactic dehydrogenase activity in the papilla tip

the papillae apices Increased LDH activity was noted in the collecting tubules of the medulla After potassium repletion the enzyme activities returned to normal.

II. Effects of Prolonged Potassium Depletion on the Kidney

The rats depleted and repleted of potassium did not develop the gross ibular changes previously described by Fourman *et al* [12] Instead, varying egrees of interstitial fibrosis were noted in the lower medullary zone of 2 per cent of the 60 rats studied over a 15-month period (Figure 8a) Mild interstitial fibrosis was found in the papillae of 35 per cent of the chronic potassium-depleted" rats (Figure 8b) No significant difference was found in their blood nonprotein nitrogen levels

Unilateral hydronephrosis was found in 8 per cent of all potassium-depleted rats depleted and repleted of potassium for periods up to 15 months (Figures 9a and 9b). The rats receiving 0 9 per cent sodium

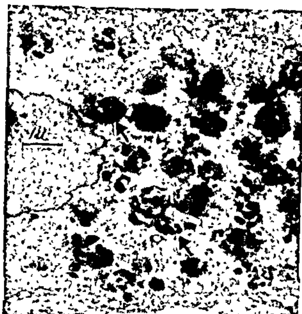


FIGURE 6b. Mitochondrial abnormalities in collecting tubules of potassium-depleted rat Electron micrograph ×10,650 This electron micrograph illustrates a variety of mitochondrial changes in the collecting tubule of a potassium-depleted rat There are dense mitochondria, lysis of mitochondria with exclusion of granules, and ghost of mitochondria Arrows point to double membrane characteristic of mitochondria

mitochondria was noted and globular bodies were seen in the cytoplasm These bodies appeared to be expelled from the abnormal mitochondria and were not similar to the huge homogeneous globules of reabsorbed protein described by Rhodin.[31]

After 5 days of potassium repletion abnormal mitochondria were fewer in number and normal mitochondria reappeared. After 10 days of potassium repletion, completely normal mitochondria were noted.

Ultramicrochemical Studies

Results of the quantitative ultramicrochemical analysis of alkaline phosphatase (APP) and lactic dehydrogenase (LDH) activity are shown in Figure 7a and Figure 7b. APP activity in the potassium-depleted animals was found to be normal in the vessels, the distal tubules, and the medullary and papillary collecting tubules Decreased APP activity was found in the glomeruli and proximal tubules and in the medullary ray of the outer medullary zone.

LDH activity was only slightly diminished in the glomeruli and proximal tubules but considerably diminished in the collecting tubules of



FIGURE 9a Normal rat kidney. This is a longitudinal cross section of a normal rat kidney. Note the thickness of the cortex and medulla



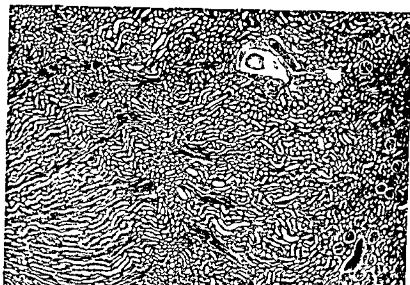
FIGURE 9b Hydronephrotic kidney of a "chronic" potassium-depleted rat Unilateral hydronephrosis was found in approximately 8 per cent of the rats depleted and repleted of potassium for periods of 15 months Note the marked dilatation of the renal pelvis and the reduced size of the cortex and medulla

chloride to drink had approximately twice the incidence of unilateral hydronephrosis as the rats receiving drinking water containing 0.45 per cent sodium chloride and 0.45 per cent sodium bicarbonate Hydronephrosis was not found in the kidneys of control animals Save for thinning of the medulla and a closer approximation of the glomeruli (Figure 9c), no obvious microscopic lesion was noted in the hydronephrotic kidney.

III. Effect of Experimental Infection on the Potassium-Depleted Kidney

Mortality Due to E. coli *Injection*

Within 24 hours after intravenous injection of *E. coli*, nine of the potassium-depleted rats died (Table IV). Five (25 per cent) were from



[illegible]

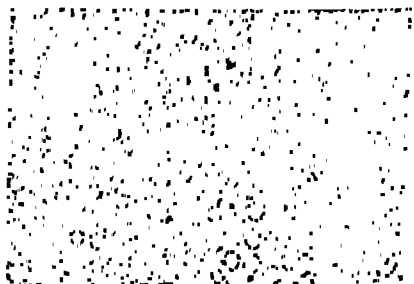


FIGURE 8b. Interstitial fibrosis of the papillae. Photomicrograph H & E ×100 Thirty-five per cent of the rats depleted and repleted of potassium for periods of 15 months developed interstitial fibrosis of the papillae

the potassium-depleted group B (injected and massaged) and four (10 per cent) were from the potassium-depleted group A (injected). These rats are believed to have died from bacteremic shock due to the gram-negative organism. Morphologic studies of their kidneys revealed no active infection. There were no immediate deaths in the control group.

An additional 24 rats died within 6 weeks following bacterial injections, 13 were potassium-depleted and 11 were controls. Except for two of the potassium-depleted group, all rats received injections of *E. coli*. Histologic findings of renal infection were present in approximately equal numbers of animals from each group (16 per cent) (Figure 10).

Pathological Studies

Gross. Gross scars of renal infection (Table V) were seen in the right kidney of four rats (45 per cent) of the potassium-deficient group B (injected and massaged). Two of the rats had scars in the unmassaged left kidney. Gross renal scars were found in three rats of the potassium-depleted group A (injected). The scars were bilateral in two rats and limited to the left kidney in one. Gross scarring was not noted in the potassium-deficient group C or the control group A_{con}, B_{con}, or C_{con}.

TABLE V. THE INCIDENCE OF RENAL SCARS IN POTASSIUM-DEPLETED RATS AND WELL-FED RATS FOLLOWING INTRAVENOUS INJECTION OF *Escherichia coli*

	Potassium-depleted Rats			Control Rats		
	Injected with *E. coli*		Injected with Broth	Injected with *E. coli*		Injected with Broth
	No Massage A	Massaged B	C	No Massage A_{con}	Massaged B_{con}	C_{con}
Gross kidney scars	13.9%	54.5%	0	0	0	0
Histologic kidney scars	10.3%	45.4%	0	0	5.9%	0
NPN mg./100 ml.	49	55	39	47	46	33

Microscopic. Microscopic studies were made of the serial sections through the renal papillae of all rats that were sacrificed. Except for one control rat of group B_{con}, microscopic scars of renal infection were limited to the kidneys of the potassium-depleted rats injected with *E. coli*. Scars of renal infection were found in four rats of potassium-deficient group B (injected and massaged). Scars were found bilaterally in two and limited to the right kidney in the other two. The majority of scars in the massaged right kidney were usually found in the renal cortex. They were multiple and usually contained calcium (Figure 11a).

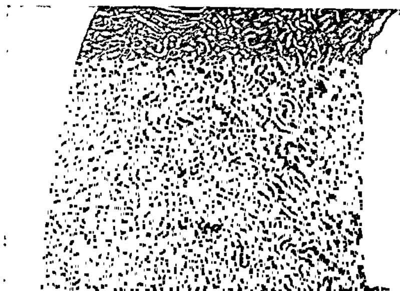


FIGURE 9C Hydronephrosis of potassium-depleted rat. Photomicrograph H & E ×55 Microscopic examination of unilateral hydronephrotic kidney of potassium-depleted rat. Note the normal-appearing kidney structure except for thinning of the cortex and medulla

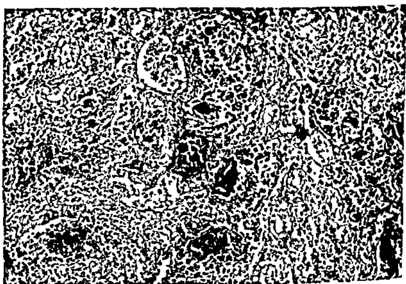


FIGURE 10 Acute renal infection in potassium-deficient rat. Photomicrograph H & E ×160. Kidney section taken from a potassium-deficient rat injected with *Escherichia coli*

TABLE VI. COLONY COUNTS GREATER THAN 100 GROWN FROM BLOOD, URINE AND TISSUES OF POTASSIUM-DEFICIENT AND WELL-FED RATS

Organ Cultured	Potassium deficient Rats								Well fed Rats						
	Injected with *E coli*							Injected with Broth C	Injected Rats						Injected with Broth C_{con}
	No Massage A			Massaged B					No Massage A_{con}			Massaged B_{con}			
Animal No	19	15	14	8	7	3	2		32	20	18	2	9	11	
Right kidney	0	+	+	+	+	+	+	0	0	0	+	+	+	0	0
Left kidney	+	+	+	+	0	0	+	0	+	+	0	+	+	+	0
Urinary bladder	+	+	0	+	+	+	0	0	0	0	+	+	0	0	0
Blood	0	0	0	+	+	0	0	0	0	0	0	+	0	0	0
Spleen	+	0	0	+	0	0	0	0	0	0	0	+	0	0	0

The potassium-deficient group A (injected) had positive *E coli* colony counts greater than 100 in the left kidney of three (10 3 per cent) rats Two of the three had this finding in both kidneys. Two rats had positive colony counts in the urinary bladder as well as the left kidney One of them also had a positive colony count of the spleen

E. coli was grown from the left kidney of three (17 6 per cent) rats of the control group B_{con} (injected and massaged) Two rats had positive *E coli* cultures grown from the massaged right kidney One of these had positive *E coli* cultures of the urinary bladder, blood, and spleen

Three (9 6 per cent) rats of control group A_{con} (injected) had positive *E coli* colony counts greater than 100 One rat had positive *E coli* cultures in both kidneys, a second had positive counts in only the left kidney, and a third had a positive *E coli* culture in the urinary bladder and nowhere else. *E coli* was not grown from blood and tissue cultures of potassium-depleted group C or the control group C_{con}

Blood Nonprotein Nitrogen

The average blood nonprotein nitrogen levels of the experimental and control animals are shown in Table V The mean values of the potassium-depleted groups A, B, and C were 49, 55, and 39 mg per 100 ml respectively. In comparison, the mean values of the control groups A_{con}, B_{con}, and C_{con} were 47, 46, and 33 mg per 100 ml respectively Statistical analysis revealed a significant difference in the NPN of the potassium-depleted *E coli* injected group A and B versus the control *E coli* injected group A_{con} and B_{con}. The P value was less than 0 0106

The blood nonprotein nitrogen of the potassium-depleted group C was significantly different from that in the control group C_{con} The P value was less than 0 0089.

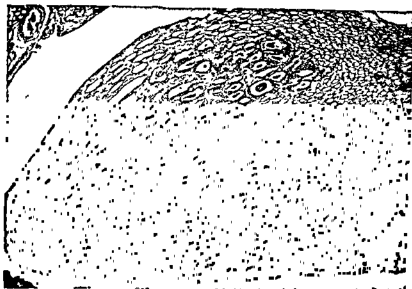


FIGURE 12b Scar of renal infection in the papillae of a potassium-depleted rat This is a kidney section of a potassium-depleted rat taken 6 weeks after injection with *Escherichia coli* Note the large scar of renal infection in the papillae Inflammatory cells are noted in the interstitial tissue and leukocyte casts in the dilated collecting tubules. *E. coli* was cultured from adjacent kidney tissue.

hyalinized while others were enveloped by periglomerular fibrosis Scars of the papillae (Figure 12b) consisted of dilated tubules with heavy infiltrates of inflammatory cells in the interstitial tissues. Many leukocyte casts were noted in the lumen of the dilated tubules.

Bacteriologic Studies

Table VI summarizes the bacterologic findings of positive *E. coli* colony counts of 100 or greater in potassium-depleted and control rats There was no statistical significant difference of positive bacteriologic cultures in the potassium-depleted rats compared with the control rats However, on rank analysis there was a significantly greater dissemination of infection in the potassium-depleted rats than the control rats.[2] The P value equaled 0.026.

Positive colony counts were observed in the massaged right kidney of four (36.3 per cent) rats of the potassium-depleted group B (injected and massaged). Two rats also had positive colony counts in the unmassaged left kidney. One of these two animals had positive cultures in the urinary bladder and spleen Three of the four rats had *E. coli* grown from the urinary bladder as well as the kidney.

rhea, primary hyperaldosteronism, or the Fanconi syndrome.[36] In 12 of these patients histologic evidence of chronic "pyelonephritis" and interstitial fibrosis was found (Table II), in an additional 5 patients only interstitial fibrosis was seen. In one patient the kidneys were hydronephrotic.

Diffuse hydropic degeneration of the convoluted tubule[1, 10] which has been considered to be pathognomonic of potassium depletion was not seen after careful search.

The model of experimental infection used to produce scars of renal infection in potassium-depleted rats certainly does not mimic the pathogenesis of "chronic pyelonephritis" in man *Nevertheless*, the interstitial fibrosis found in the kidneys of rats chronically depleted of potassium is similar to the interstitial fibrosis so commonly found in patients depleted of potassium for longer periods.

SUMMARY

(1) "Chronic pyelonephritis" was commonly found in the kidneys of patients with prolonged potassium deficiency

(2) Structural and biochemical abnormalities were produced in rats made deficient of potassium for short and prolonged periods

(3) Mitochondrial abnormalities were found limited to the collecting tubular cells of the papillae apices of potassium-depleted rats These abnormalities were associated with a reduction of lactic dehydrogenase activity.

(4) Rats with prolonged potassium deficiency had developed interstitial fibrosis of the inner medullary zone, the papillae, or both. In addition, approximately 8 per cent of the rats depleted of potassium for prolonged periods had unilateral hydronephrosis

(5) Studies of experimental infection on the kidney of potassium-depleted animals indicated that the potassium-depleted kidney was more susceptible to renal infection than the kidney of well-fed rats

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Comments

These investigations re-emphasize the well-known fact that when an animal was depleted of potassium, structural and biochemical abnormalities developed in the kidney. In acute potassium depletion, reversible lesions appeared and were limited to the mitochondria of the collecting tubules of the papilla apex. *Moreover, those morphologic changes were* associated with cytochemical abnormalities such as the reduction of lactic dehydrogenase activity. In chronic potassium depletion, irreversible changes of interstitial fibrosis appeared in either the papillae tips, the inner medullary zone, or both.

When these structural and biochemical changes in the renal papillae were challenged by experimental infection, the rats in acute and chronic potassium depletion were found to be more susceptible to renal infection. For example, a significantly greater number of potassium-depleted rats developed renal scars after infection, and a significantly greater dissemination of positive bacteriologic cultures was found after *E. coli* injection than in the controls. The wedge-shaped scars seen in the kidney of potassium-depleted rats appeared to have developed in the papillae and extended to the cortex. This suggests that the structural and biochemical papillary defects might serve as a *locus minoris resistantiae*.

According to Jackson[15] the *E. coli* strain 0.22, K-negative had three distinct characteristics that modified the model of experimental infection First the organism produced an endotoxin which caused endotoxic shock and killed 15 per cent of the potassium-depleted rats within 24 hours *following injection Endotoxin is known to produce biochemical* abnormalities in the kidney[36] which may also increase its susceptibility to infection. In this regard, a diffuse severe and acute necrotizing "pyelonephritis" was found on morphologic study of the kidneys of rats that died several days after bacterial inoculation.

Jackson[15] also states that the organism used is capable of producing a high percentage of bilateral renal affliction

In our study a high percentage of potassium-depleted animals were noted to have bilateral scars following infection as well as positive cultures of *E. coli* from both kidneys.

The organism used had a proliferative growth phase in the blood stream between the fortieth and forty-fourth days after inoculation.[15] This bacteriologic characteristic may explain our finding of positive bacteriologic cultures from the spleen and blood at the forty-second day after *E. coli* injection.

With regard to man, a study was made of kidney tissue removed by biopsy from 18 patients with prolonged potassium depletion due to diar-

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As to the question of the occurrence of fibrosis after the repletion of the animal with potassium, our findings agree with Dr Muehrcke's in that it is present but minimal This agreement conflicts with the earlier experiments of McCance, who described extreme fibrosis and the production of what might be called an irreversible contracted fibrotic kidney, from which no restitution would seem possible But here again there is something we do not fully understand because in some potassium-depletion experiments done by Drs Welt and Holliday several years before the present series was started, something analogous to the McCance description was observed, we have never been able to reproduce it, however

In considering these renal lesions I think we must keep in mind that depletion of potassium in an animal upsets the entire electrolyte balance of the tissue and body fluids, and the final picture in the kidney may be influenced by other electrolyte and protein intakes and these vary in different experimental procedures The degree of alkalosis, the chloride or phosphate load or that of calcium, all seem to alter the character of the renal lesion, so that although potassium depletion triggers the disturbance and repletion of potassium largely corrects the situation, the renal effects can hardly be described as the result of a simple deficiency in potassium.

Another confusing element in the study of the renal effects of potassium depletion is the rather remarkable species differences that are observed You are all familiar with the peculiar clean-cut vacuoles described by Drs Relman and Schwartz in the proximal convolutions of human kidneys from clinical cases of potassium deficiency I have never seen this distinctive histologic picture in the rat kidney, nor am I familiar with any published photographs which show it I have looked at eight human cases whose serum potassium was low over a considerable period and found the vacuoles in six, but in only one of these were there droplets in the collecting tubules and in none was the hyperplasia of intercalated cells which is so remarkable in rats noticeable In fact the collecting system in all but the one might be described as structurally normal, yet failure of concentration is considered typical of the human renal effect, so in the one species we have agreement between structure and function, in the other not. Dr. Segar of Indianapolis has recently sent me a kidney of one potassium-depleted monkey, the sole cooperator in the original group of six, the renal lesions are similar to those in the rat I have only seen two kidneys from depleted dogs, they showed little if any structural changes.

To sum up it does not seem to me remarkable that the descriptions of the effects of potassium depletion vary considerably, since this can be explained by the fact that taking away potassium from an animal does much more than deplete him of potassium The differing species results observed in the kidney may be due to different sorts of electrolyte

GENERAL DISCUSSION

Dr. Kiley I should like to ask Dr. Muehrcke to comment further on ureteral function in chronic potassium depletion.

Dr. Muehrcke We have not specifically studied the tone of the ureter in potassium deficiency. In the rats that were depleted and repleted of potassium for a period of fifteen months, we noticed that approximately 8 per cent of the animals had hydronephrosis This hydronephrosis on microscopic examination was very similar to the human counterpart. Scars of infection or fibrosis were not found. There was a thinning of the medulla and cortex, associated with a close approximation of the glomeruli. These morphologic findings were observed in the kidney of one patient who was chronically depleted of potassium.

Dr Woods Drs. Welt, Hollander, Newton, and I have recently investigated this problem and found that rats with renal lesions resulting from a previous episode of potassium depletion exhibit increased susceptibility to experimental pyelonephritis. The susceptibility of rats during *acute* potassium nephropathy was also studied, but since the number of animals was small and the results inconclusive, this problem is being made the subject of further investigation

Dr. Oliver As a pathologist I never supposed that I would get involved in renal lesions that had anything to do with electrolytes, because they seemed to me to be something that I surely could not see and therefore I should have nothing to do with them

My friends Drs. Welt, Hollander, and Holliday, however, did get me involved, and so I have looked at potassium-depleted rats under all conceivable variations of experimental procedure I think our findings agree quite well with what Dr Muehrcke has described

The droplets in the collecting tubules of the potassium-depleted rat are peculiar configurations and considerable mystery remains as to their origin and significance. In some unpublished experiments we find they react quite differently to vital staining with Evans blue from the protein absorption droplets that occur in the proximal convolutions under certain conditions They are localized in a very unusual situation, the pelvic epithelium and the larger collecting ducts; they are rarely seen in the outer zone of the medulla where the hyperplasia of intercalated cells is marked Thus the entire collecting system of the rat is altered by potassium depletion, but by two very different sorts of lesions, each of which is sharply limited to a specific zone of the medulla

problem I might say that there is an unpublished paper by one of the Boston groups describing experiments in which they tied off the renal vein in part — almost the opposite of the Goldblatt situation — and presumably produced polycythemia in about seven out of ten situations. That is the only thing I know of. That paper has not been published, and I have not seen the data

DR MUEHRCKE: When one speaks of potassium deficiency in rats or in man, one should consider that the tissues of animal or man could be deficient in potassium but the kidney may react in several ways to potassium deficiency. For example, if the potassium deficiency was caused by a lack of potassium in the diet or by gastrointestinal loss, the kidney will conserve potassium.

On the other hand, patients with primary hyperaldosteronism will lose tremendous quantities of potassium through the kidney into the urine In this instance we have a kidney in a potassium-depleted individual but the kidney is losing potassium.

In future studies we should differentiate the type of potassium-depleted kidney we are studying. Potassium depletion in the 18 patients reported in the previous study was due to a variety of causes Some had potassium-conserving kidneys where the chronic potassium depletion was due to gastrointestinal loss of potassium Others had potassium loss through the kidneys into the urine due to primary hyperaldosteronism, steroids, diuretics, or tubular defects

The histologic criteria of chronic pyelonephritis in man are very similar to those that Dr. Pirani described last evening These were interstitial fibrosis, cellular infiltrates, periglomerular fibrosis, and tubular dilatation In some cases the glomeruli were completely hyalinized, and where the nephron was abnormal marked tubular dilatation was seen These tubules contained colloid casts.

DR. CARONE: Drs. Epstein, Kashgarian, and I have been interested in the subject of potassium deficiency and pyelonephritis Our experiments were acute and we did not massage the kidneys. We found that potassium-deficient rats injected with *Escherichia coli* showed no significant incidence of pyelonephritis This was also true in mice which were rendered potassium-deficient.

The strain of *E. coli* that we used is one which normally does not produce pyelonephritis in the unobstructed rat kidney. We know that it had a low virulence for the rat kidney because it did not produce significant pyelonephritis in kidneys damaged with mercuric chloride or uric acid, or in kidneys with nephrocalcinosis.

For this reason we decided to switch to an organism which does pro-

or metabolic imbalance that occur in the given species, or it may be that the kidneys of the various species vary in their response to similar metabolic situations.

Dr. McDonald Dr. Jacobson, have you had an opportunity to show that supplemental administration of erythropoietin to azotemic patients with defective kidney function can correct the anemia? Also, I should like to ask Dr. Boyce whether he believes that the determination of bound glucose might be a practical way of determining whether or not pyelonephritis is present in the presence of bacteriuria.

Dr. Jacobson Giving erythropoietin to a patient has been possible only in the last year or so, because no amount of erythropoietin has been made available that will give this opportunity. The only thing I can say about that is that Dr. Castle and his associates some years ago were able partially to correct the anemia of nephritis with the use of cobalt, and of course cobalt specifically elevates the erythropoietin titer. So this is indirect evidence that it probably would do some good.

Dr. Boyce: I wish to thank Dr. McDonald for giving me a chance to emphasize a point which I am sure he recognized. We feel very strongly that determinations of sialic acid or hexose or hexosamine or any other of the moieties of mucosubstances which are common to more than one are very shaky grounds as an index to the presence of any specific component. For example, sialic acid is one of the common terminal groups of many mucosubstances, and it is also one of the most labile of such groups To utilize any such moiety as an estimate of specific substances present in a mixture is quite unreliable. For this reason we like to go back to the old-fashioned method of weight as the comparative basis instead of using comparative methods involving various terminal group substances only. I don't think pyelonephritis can be diagnosed by the amount of hexose in the urine.

Dr. Hollander: I should like to ask if Dr. Jacobson or anyone else studying the problem has been able to extract hemopoietin from the kidneys or identify it in higher concentration in renal venous blood

I wonder if Dr. Muehrcke would tell us a little more about the diagnostic criteria for pyelonephritis in his human cases, since this is of obvious interest to this conference and is a matter on which there is considerable disagreement.

Dr. Jacobson: We have made some attempts to get material from the kidney in the normal animal as well as in kidneys of animals that have been subjected to high altitude, and have only equivocal data on this

to the functional defect. Secondly, we have seen in examining biopsy specimens that the vacuolar nephropathy may not be diffuse. In some specimens it is quite diffuse, but in some specimens one will see areas of vacuolar nephropathy in among large areas of apparently normal epithelium

DR. MUEHRCKE: I should like to clarify our definition of potassium deficiency. These patients had persistent hypokalemia In some of these patients metabolic studies showed them to be depleted of as much potassium as 1000 mEq.

There are biochemical changes that accompany structural changes in the potassium-depleted kidney. Using Bonting's technique, which is a modification of Lowry's, we found increases of lactic dehydrogenase activity in the collecting tubules of the papilla apex

I wish to comment on the excellent paper presented by Dr Freedman He gave his rats ammonium chloride for eight or ten days This is one method of producing potassium depletion in animals He observed the same biochemical changes in his rat kidneys that others found in potassium-depleted rats.

First, there is an increase of glutaminase activity in the convoluted tubules Second, the cellular pH drops, as in the rats he fed ammonium chloride Third, in potassium depletion a large percentage of the arginine that is excreted in the urine is excreted in the form of ammonia, very much as Dr. Freedman reported

Going back to our human material and to answer Dr Relman's questions, our potassium-depleted patients had renal functional effects that mimic very closely those he described These were a fixed specific gravity and proteinuria from a trace to 2 plus Serial renal biopsies were done on the patients during potassium depletion and after repletion with potassium We were unable to see any significant difference in the kidney tubules as to the presence or absence of vacuoles After following them for four or five years, we have observed the development of histologic evidence of chronic pyelonephritis Cultures of their kidney tissues were sterile One of our patients with primary hyperaldosteronism had been depleted of potassium for twelve years She had three episodes of acute pyelonephritis during this illness

SUMMATION

MAURICE B STRAUSS

(Boston, Massachusetts)

The shortness of the time remaining comes as a boon to me because I wish to limit my remarks to nephrogenic hypertension

duce pyelonephritis in the normal rat kidney. For this purpose we used an enterococcus. We adjusted our dose so that it produced pyelonephritis in approximately 15 per cent of normals. With this organism we found that the incidence of pyelonephritis in potassium-depleted rats was approximately three times normal. However, we also found that these animals were developing infections elsewhere in mediastinal structures and in abdominal tissues, and that the infection in these sites other than the kidney was usually marked compared with the degree of infection in the kidney. For this reason we felt that potassium deficiency increases the susceptibility to enterococcal infection in general with no specific effect upon the kidney.

In many of our potassium-depleted rats we saw interstitial fibrosis and tubular obstruction in the collecting ducts with tubular dilatation proximally. In many cases there was some round cell infiltration These kidneys were negative bacteriologically and showed no evidence of acute inflammatory response.

In the experiments of Fourman and McCance, they repleted their animals and found that the severe changes of scarring and atrophy were progressive and not reversible. I spoke to Dr. Fourman about this and wondered why some other investigators could not repeat his results He felt that this was due to failure to take into account the original weight of the animals when the experiments were initiated.

Dr. Relman. Dr. William Schwartz and I have observed that in potassium-depleted patients one will find the functional defects characteristic of potassium depletion in some patients who do not have any vacuoles on biopsy. We do not have a wide enough experience with histologic material to be able to have any idea as to how frequently, in a large group of patients with potassium depletion and the functional defect, one might expect to find vacuolar changes. So in one sense I am not surprised to hear that Dr. Muehrcke has found *some* potassium-deficient patients in whom he did not find vacuolar nephropathy. However, I am a little surprised to see that in *none* of his patients did he see any vacuoles.

I should therefore like to ask him two questions. First, was he able to *demonstrate that in every one of these patients there* were unequivocal functional defects characteristic of potassium depletion which could be reversed by curing the potassium depletion? Second, did he have an opportunity to obtain any serial biopsies in these patients?

I ask the latter question for two reasons. First, because sometimes in the absence of vacuolar nephropathy there will be very minimal changes in the cytoplasm of the proximal tubular epithelium, which are quite nonspecific. Unless one has a chance to see these changes disappear with *treatment*, one can never be sure that they had any relationship at all

VII

Treatment of Pyelonephritis

Chairman: MAXWELL FINLAND, M.D *(Boston, Massachusetts)*

In considering experimental nephrogenic hypertension, I think one must give thought to a former student at the Hopkins Medical School who attended for four years (but who did not graduate) — Gertrude Stein. Thus, concerning nephrogenic hypertension, we can say that man is not a dog, is not a rat, is not a rabbit, is not a cat, is not a giraffe.

There are certain facts, however, that seem to me to stand out concerning nephrogenic hypertension in man. The presence of one normal kidney does not prevent the occurrence of hypertension under certain circumstances when the other kidney is diseased. The removal of the diseased kidney, whether the disease appears to be primarily of vascular origin or primarily the result of infection, may be followed by lasting relief of the hypertension.

These facts I think are clear, and to me seem to establish a relationship between the kidney and hypertension at least under these circumstances

As for the concept that the failure of the kidney to excrete a vasoconstrictive substance constitutes a substantial factor in nephrogenic hypertension, we must recall that individuals with acute tubular necrosis and anuria, provided they are not overloaded with salt and water, may have anuria for three to four weeks without hypertension ensuing

The suggestion was made in one of the papers this morning that hypertension can be related to a renoprival factor, a diminution in renal mass. Dr. John Merrill has given me permission to quote some of his recent observations He observed four patients in whom all renal tissue was surgically removed. Provided they were not overloaded with sodium and water, they maintained normal arterial blood pressures during periods of from two weeks to slightly over two months. These observations appear to me to eliminate the need for the concept of renoprival hypertension in man.

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*Antibiotics as Inhibitors of Bacte ial Cell Wall Synthesis**

JACK L STROMINGER, M D

(St. Louis, Missouri)

In recent years it has become apparent that a number o t ıctenal substances owe their selective toxicity for microorganisms ı ı ıhibition in some manner, of bacterial cell wall synthesis Anım s do not possess structures chemically or morphologically equiv ıl ı cterial cell walls, and hence the selective toxicity of these su ı ue to interference in a metabolic sequence uniquely found ın ı acterial cell.

To explain the mechanism of this inhibition in det il I sh introduce my subject by discussing briefly what is known ıb t the nature of bacterial cell walls and about the mechanisms wh ı many organisms possess for the synthesis of complex molecules Bacterial cell walls are heteropolymeric substances in that they contain within a single organic molecule amino acids which are constituents of proteins, sugars which are constituents of polysaccharides, and a polyalcohol phosphate, ribitol phosphate, which is related to the phospholipids Each of these types of compounds is synthesized biologically through the activation of its small components as nucleotides. Thus, amino acids are activated for protein synthesis by means of a derivative of the nucleotide, adenylic acid The alcohol, choline, is activated for phospholipid synthesis by means of a derivative of the nucleotide, cytidine ıhosphate, and sugars, for example glucose, may ı ıd for ıccharide synthesis by means of a derivative of the de, ı hosphate Each of these types of mechanisms is r ı the ı of the cell wall. However, since I shall deal m ı th ı uridine nucleotides in the process, I want to ı discussion by outlining what in general is know ı e nucl ıdes in polysaccharide synthesis.

*Supported by ı Allergy and Infectious Diseases (E-1902 ı d from the National Science Foundation

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*Antibiotics as Inhibitors of Bacterial Cell Wall Synthesis**

JACK L. STROMINGER, M.D.

(St Louis, Missouri)

In recent years it has become apparent that a number of antibacterial substances owe their selective toxicity for microorganisms to inhibition, in some manner, of bacterial cell wall synthesis. Animal cells do not possess structures chemically or morphologically equivalent to bacterial cell walls, and hence the selective toxicity of these substances is due to interference in a metabolic sequence uniquely found in the bacterial cell

To explain the mechanism of this inhibition in detail, I shall introduce my subject by discussing briefly what is known about the nature of bacterial cell walls and about the mechanisms which many organisms possess for the synthesis of complex molecules Bacterial cell walls are heteropolymeric substances in that they contain within a single organic molecule amino acids which are constituents of proteins, sugars which are constituents of polysaccharides, and a polyalcohol phosphate, ribitol phosphate, which is related to the phospholipids Each of these types of compour [illegible]
compone [illegible]
synthesis [illegible]
alcohol, [illegible] is activated [illegible]
derivative of the nucleotide, cytidine diphosphate; and sugars, for example gluc [illegible]
of a derivat [illegible]
of mechanisms is involved in the [illegible] of uridine nucleotides in the
since I shall deal mostly with the role of uridine nucleotides in the process, I want to provide a background for discussion by outlining what in general is known about the role of uridine nucleotides in polysaccharide synthesis

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A typical uridine nucleotide contains uridine linked through a pyrophosphate bridge to a sugar. The sugar may be glucose, acetylglucosamine, or any of a number of other substances One may consider that the sugar is a *fragment* which is activated by the nucleotide for a synthetic reaction. There are several groups of compounds which are activated in this manner. One group of compounds is related to UDP-glucose and includes UDP-glucuronic acid, UDP-galactose and UDP-galacturonic acid. Once the sugar is activated in the form of its nucleotide derivative, it can be used in a number of different reactions. For example, the syntheses of sucrose, glucuronides, lactose, and probably also of pectin are mediated in this manner.

The reactions which lead to the synthesis of glucuronides provide an example of a typical reaction mechanism which results in such a synthesis Many drugs, other foreign organic compounds, and natural substrates are detoxified through this mechanism First, the sugar is activated as UDP-glucose through a reaction between UTP and glucose-1-phosphate. Next, the glucose moiety of the nucleotide is modified by oxidation to glucuronic acid, and then the nucleotide can transfer its glucuronic acid moiety to some acceptor with the formation of a glucuronide. Uridine diphosphate, the second product of this reaction, can then be converted to UTP by phosphorylation and participate again in the synthesis of another mole of the glucuronide. What this representation (Figure 1) emphasizes is that uridine nucleotides act catalytically as carriers of sugar fragments for synthetic reactions.

Another group of compounds which are activated in this manner includes compounds related to UDP-acetylglucosamine. Several of these compounds are intermediates in mucopolysaccharide synthesis Other compounds related to UDP-acetylglucosamine are intermediates in the synthesis of the bacterial cell wall.

There are several ways of defining bacterial cell walls For example, Victoria blue is a dye which stains deeply an internal structure of the microorganism, termed the cytoplasmic membrane. External to the cytoplasmic membrane and best seen at the ends of cells treated with Victoria blue is another structure, termed the cell wall, which is poorly stained by the dye. The cell wall lies between the capsule and the cytoplasmic membrane in encapsulated bacteria.

The cell wall can also be defined physiologically. It has long been known that a sensitive bacterium, for example *Bacillus megaterium*, can be lysed by enzymes, termed lysozymes, which are found in egg white, tears, and leukocytes, among other places. However, Weibull observed that if the treatment with the enzyme was carried out in a medium containing hypertonic sucrose, the organisms were not lysed, but instead the rod-like forms were converted by the enzyme into spherical forms,

which Weibull termed protoplasts. The action of lysozymes is to digest the bacterial cell wall, and these experiments then define the cell wall as the rigid external structure which gives form and shape to the microorganism and serves to protect it from such deleterious influences as osmotic shock. Electron micrographs of a cell wall preparation from

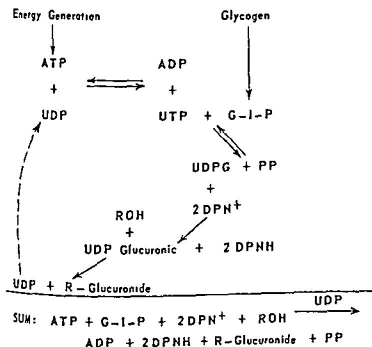


FIGURE 1. The over-all reaction in glucuronide synthesis a UDP cycle This representation emphasizes that uridine nucleotides act catalytically in the synthesis, and that ATP is the source of the energy employed.

B megaterium show that the cell wall, like the organism itself, has a sausage-like shape On the other
aureus show that the cell wall o
has a globoid shape Again this
rigidity and the shape which are characteristic of the microorganism

Two lines of investigation led independently to the conclusion that penicillin inhibited bacterial growth and multiplication by inhibiting in some manner cell wall synthesis It had been observed by Liebermeister and Kellenberger that spherical forms gradually developed in a culture of *Bacillus proteus* under the influence of penicillin This phenomenon

was also observed by Lederberg working with *Escherichia coli* in broths containing hypertonic sucrose. Lederberg recognized that these forms were analogous to the protoplasts which are formed by digestion of *B. megaterium* with lysozymes. He therefore inferred that penicillin must be interfering either with the synthesis of the cell wall or with its maintenance.

A second line of investigation which led independently to the same conclusion began with the observations of Park and Johnson that uridine nucleotides accumulated in staphylococci inhibited by penicillin The structure of one of these nucleotides which accumulate is shown in Figure 2. It contains uridine linked through a pyrophosphate bridge to a

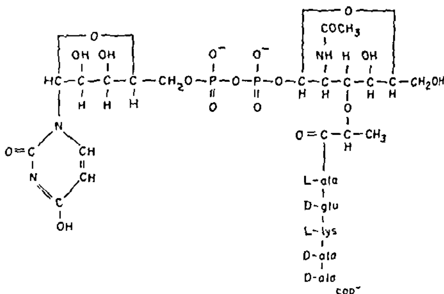


FIGURE 2 The structure of a uridine nucleotide which accumulates in penicillin-treated *Staphylococcus aureus*

sugar. At the time the compound was isolated the sugar was known to be a very unusual substance, but its structure could not be deduced Only recently has the sugar been shown conclusively to be a lactic acid ether of acetylglucosamine. It has been given the trivial name, acetylmuramic acid. The most complex compound which accumulates contains a peptide attached to the lactic acid moiety. The sequence of this peptide is (L)alanyl-(D)glutamyl-(L)lysyl-(D)alanyl-(D)alanine. The occurrence of the dipeptide, (D)alanyl-(D)alanine, in the C-terminal position is a point to which we shall return in a few moments. The occurrence of

(D) amino acids in the peptide, a form of amino acids which does not occur in proteins, is another unusual feature of the structure

At the time this compound was isolated no guess could be made a to what function it might have However, in the following years a great deal of work was done on the structure of bacterial cells walls To sum it up, it has become apparent that all bacterial cell walls have a basal structure, which contains two sugars, acetylglucosamine and a lactic acid ether of acetylglucosamine, and three amino acids, alanine, glutamic acid, and either lysine or diaminopimelic acid The latter is another unique microbial structure These and other qualitative observations suggested that the cell walls had a striking relationship to the uridine nucleotides which accumulated Careful quantitative analyses of the cell walls from two strains of staphylococci which accumulated the nucleotide were therefore carried out The ratio of amino acids in the wall was almost identical with the ratio in the nucleotide, and both the nucleotide and the wall contained one mole of the sugar per mole of the peptide fragment The comparison was even more striking when the optical rotations of the amino acids were compared In the wall as in the nucleotide there were two (D)alanine residues for each (L)alanine residue All of the glutamic acid was (D)glutamic acid, and all of the lysine was (L)lysine This striking structural similarity suggested that the nucleotide is a precursor of the cell wall and that its accumulation in the penicillin-inhibited cell was the consequence of inhibition of cell wall synthesis by the antibiotic

This inference is now supported by direct isotopic measurements of the rate of cell wall synthesis (Table I) In *S aureus*, under the influence

TABLE I Effects of Penicillin on Incorporation of Isotopes into Cell Wall or into Cell Protein and Nucleic Acid in *Staphylococcus aureus* and in *Escherichia coli*

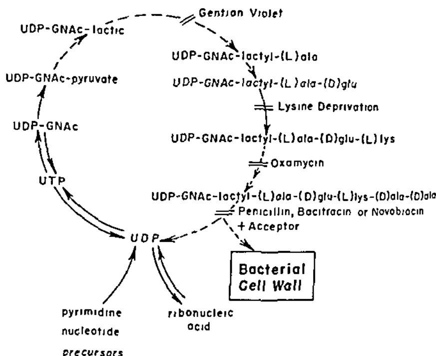
Isotope	*Staphylococcus aureus*				*Escherichia coli*
	C^{14} lysine		P^{32} inorganic phosphate		H^3 DAP
	cell wall	protein	cell wall	nucleic acid	cell wall
Control	34,800	5,100	155,000	11 600	1 040 000
+ Penicillin	3,290	4,960	48,900	11 600	297,000
% inhibition	91%	2%	68%	0	72%

Data are expressed as specific activities (c p m mg)

of penicillin, the rate of incorporation of C^{14}-lysine into the cell wall was diminished by 91 per cent under conditions where there was little or no inhibition of incorporation of the isotope into cell protein Thus, the effect of penicillin on cell wall synthesis is a relatively specific one Similar data were obtained with *E. coli* when care was taken to utilize an isotope

(tritium-labeled diaminopimelic acid) which is a specific precursor of the basal structure of the cell wall.

The information which is now available about the mechanism of cell wall synthesis is summarized in Figure 3. It is analogous to the mechanism of glucuronide synthesis. First a sugar, acetylglucosamine, is activated as



... of part of the cell

representation does not imply that these substances specifically inhibit the indicated reactions

a uridine nucleotide. Then it is modified by the stepwise addition of lactic acid and of amino acids to form the acetylmuramic acid-peptide fragment on the nucleotide. Finally, this fragment is presumably transferred to the cell wall, although this transfer has not yet been directly demonstrated. Most of the other reactions shown in this cycle have now been studied as reactions catalyzed by purified enzymes, but that information is beyond the scope of this paper.

Penicillin, bacitracin, and novobiocin all inhibit the cycle at a late point, leading to the accumulation of a late intermediate. Because the

transfer reaction has not yet been measured, little is known about the mechanism by which these three substances block the cycle. On the other hand, oxamycin inhibits the cycle at an earlier point, leading to the accumulation of an earlier intermediate. The precise mechanism by which this occurs has been worked out. Deprivation of lysine or treatment with a dye closely related to gentian violet inhibits the cycle at still earlier points, leading to the accumulation of earlier intermediates in the reaction mechanism.

Similar intermediates also occur in the synthesis of the basal structure of the gram-negative bacillus, *E. coli*. Penicillin at appropriate concentrations may inhibit the synthesis of the cell wall of *E. coli* in a manner analogous to the inhibition of synthesis of the cell wall of *S. aureus*. The relative insensitivity of *E. coli* to penicillin might be the consequence of inability of this antibiotic at low concentration to penetrate through the extremely complex cell wall of this microorganism.

I shall devote the rest of this paper to describing in some detail the mechanism by which oxamycin inhibits cell wall synthesis. It was observed that one of the nucleotides which accumulated in the presence of oxamycin had a slightly different position on a two-dimensional paper chromatogram than one of the compounds which accumulated in the presence of penicillin. This compound was isolated and its structure was determined. It may be represented as UDP-GNAc-lactyl-(L)ala-(D)glu-(L)lys. It contains one rather than three alanine residues, and is missing the two terminal (D)alanine residues from the peptide sequence. This suggested that the antibiotic might be a competitive inhibitor of the incorporation of (D)alanine into the nucleotide. This was readily demonstrated. The accumulation of nucleotides induced by oxamycin was progressively reduced by increasing amounts of (D)alanine. (L)alanine was completely ineffective in preventing the accumulation of nucleotides induced by oxamycin. Not only could (D)alanine prevent nucleotide accumulation by oxamycin, but it could also reverse nucleotide accumulation previously induced by oxamycin. The cells accumulated 20 micromoles of nucleotide during 45 minutes of incubation with oxamycin. If incubation was continued 45 minutes longer with no further addition, the amount of nucleotide increased to 30 micromoles. If, on the other hand, (D)alanine was added during the second incubation, the amount of nucleotide was reduced to 12 micromoles, or to 7 micromoles with a larger amount of (D)alanine. Again, (L)alanine and several other substances were completely ineffective in replacing (D)alanine as a competitor for oxamycin. A plot of the reciprocal of the velocity of nucleotide accumulation as a function of the reciprocal of the concentration of the antibiotic at different concentrations of (D)alanine (according to the method of Lineweaver and Burke) indicated that the data fit the

but special interest in the inhibition of alanine racemase and of inhibition of the dipeptide synthesizing enzyme by the antibiotic lies in the fact that these reactions are uniquely found in bacterial cells, and hence the substance is a useful antibacterial agent. The principle illustrated may be useful in the possible rational design of chemotherapeutic agents, a possibility which does not seem so far away as it did a few years ago. Chemical approaches to the mechanism of action of antibiotics may also yield new information about the ways in which cells become resistant to these agents Furthermore, it is apparent that the combination of antibody with complement, which leads to the inactivation of the bacterial cell, is, after all, a reaction of an antibody to a surface antigen, located in the cell wall Some of the defense mechanisms of the host may, therefore, also be directed at the integrity of the cell wall. With reference to the kidney in particular it seems possible that the hypertonicity of the renal medulla could play some part in preserving the viability of microorganisms located there in the face of attack by antibiotics and by these defense mechanisms

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Analysis of the Restrictions Imposed by Renal Failure on Antibiotic Therapy of Pyelonephritis

CALVIN M KUNIN, M.D.
(Charlottesville, Virginia)

The treatment of an acute episode of uncomplicated pyelonephritis in which the offending organism is *Escherichia coli* is rather simple These infections usually respond to measures such as acidification of the urine, or administration of any one of a number of organic acids, or sulfonamides or antibiotics effective against gram-negative organisms More complicated infections, in which structural derangement of the urinary tract architecture has occurred and in which the invading organisms appear to be more resistant to therapy, present a formidable therapeutic challenge[3] When renal failure is superimposed upon the latter difficulties, therapy is not only more difficult but may, in itself, be deleterious to the patient

The antibiotics in common use are not entirely innocuous agents, particularly when used in large doses for prolonged periods of time Liver and renal damage, negative nitrogen balance, and increased excretion of riboflavin and of certain vitamins and amino acids into the urine may occur in patients treated with any of the tetracyclines Renal damage has been noted with the use of bacitracin, the polymyxins, neomycin, and kanamycin. Damage to vestibular or auditory functions occurs with streptomycin and dihydrostreptomycin, neomycin, kanamycin, and vancomycin, and bone marrow depression and the so-called "gray syndrome" (cardiovascular collapse) in infants have occurred in association with chloramphenicol therapy. These adverse effects of antibiotics have been recently reviewed[6] It is therefore important to use these drugs in such a manner that maximum therapeutic advantage is obtained with a minimum of toxic hazard This is particularly significant in the situation in which an acute flare-up of pyelonephritis precipitates severe renal decompensation In the presence of uremia ordinary therapeutic doses of antibiotics as well as other medicaments may actually be excessive and drug toxicity may become life-threatening

In this presentation we hope to outline some of the principles which may guide the therapist in the use of antibiotics for the treatment of urinary tract infections in uremic patients. We do not presume to answer

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the question regarding the relative importance of high tissue levels of drug versus high urine levels. This problem becomes moot for the uremic patient in whom the underlying structural and functional damage themselves limit tissue and urine levels of drug.

Factors Which Influence the Rate of Removal of Antibiotics from the Body

Certain physical and chemical characteristics of each drug determine the rate at which it will be removed from the body or inactivated *in vivo.* Some of these properties are (1) inherent stability, (2) mode and extent of renal removal, (3) extent of plasma protein binding, (4) other modes of excretion (through conjugation, excretion into the bile and gastrointestinal tract), (5) sequestration and release from various organs such as the liver.

Table I contains a summary of some of the above properties for the antibiotics in common use with particular regard to factors (2) and (3) In addition, information is presented concerning the half-life in serum of each drug (where available) following a single injection given to normal and anuric patients. Finally, the amount of active drug recoverable in the urine is included to provide some measure of the inherent stability of each drug and relative importance of the kidney in its removal. It should be noted that antibiotics even in the same chemical group (the tetracyclines) differ markedly in these pharmacologic properties, so that each must be considered individually. This matter has been recently reviewed in detail elsewhere[7]

Control of the Concentration of Antibiotics in the Blood

With the exception of penicillin and the metabolic products of chloramphenicol (chiefly the monoglucuronide), which are excreted largely by active tubular transport, the antibiotics in current use are cleared by the kidney by glomerular filtration. The rate at which each antibiotic is cleared is dependent upon the extent to which it is bound to plasma proteins (see Table I). Some of the antibiotics are metabolized or excreted by other channels at a rate so rapid that the contribution of the renal clearance may be almost negligible (active chloramphenicol, erythromycin, novobiocin, and chlortetracycline). Others are excreted into the urine largely unchanged and will potentially tend to be retained in the presence of renal failure (streptomycin, tetracycline, oxytetracycline, demethylchlortetracycline, bacitracin, kanamycin, neomycin, polymyxin

[...]ERMINE THE PERSISTENCE OF ANTIBIOTICS IN PATIENTS WITH RENAL DISEASE *

Antibiotic	Renal Clearance †		Per Cent of Parenteral Dose Recovered in the Urine	Per Cent Bound to Plasma Proteins	Half-life in Serum	
	Rate	Probable Mechanism			Normal Subjects	Anuric Patients
					hr	hr.
Penicillin G	560–1080 ml/min	G + T‡	58–85	48–58	0.5§	7.2–10.5
Streptomycin	30–70 ml/min	G	30–80	34	2.4–2.7	52–110
Tetracycline	62% of Cl_{creat} ‖	G	60	24	8.5	57–108
Chlortetracycline	30–37% of Cl_{creat}	G	18	47–70	5.6	6.8–11.0
Oxytetracycline	85% of Cl_{creat}	G	70	20	9.6	—
Demethylchlortetracycline	30% of Cl_{creat}	G	42	41	12.3	—
Chloramphenicol active	24 ml/min	G	5–15	60	1.6–3.3	3.2–4.3
Inactive conjugates	340 ml/min	I	70–80	?	3.7–4.6	68.5–154.3
Erythromycin	76% of Cl_{creat} (dogs)	G	15	?	1.4	4.8–5.8
Novobiocin	low	G	15–35	95	2.3	—
Bacitracin	159 ml/min	?G	9–30	?	1.5§	—
Kanamycin	81–150% of Cl_{creat}	G + ?I	78.4–118.5	low	3§	—
Neomycin	?	G	52–90	low	3§	—
Polymyxin B	?	?G	30–50	?	6§	—
Vancomycin	69% of Cl_{creat} (dogs)	G	large	?	6§	—
Ristocetin	67% of Cl_{creat} (dogs)	G	30–40	?	?	—
			85–100			
			79.4 (dogs)			

Note References to these data are presented elsewhere (see reference 8)

* All data are for man except where otherwise specified

† Divergent values for renal clearance and "half life" were reported by Spitzy and Hitzenberger [9] for most of the antibiotics listed and they are not included in this table

‡ G = glomerular filtration, T = tubular excretion

§ Approximate values

‖ Cl_{creat} = creatinine clearance

B, vancomycin, and ristocetin). Penicillin lies somewhere between these two groups, being rapidly excreted into the urine but also rapidly inactivated *in vivo*, probably by the liver.[6]

The serum half-life of each antibiotic reflects the rate of both renal and nonrenal clearance mechanisms. Figure 1 depicts a hypothetical representation of the relation between renal function and the half-life of anti-

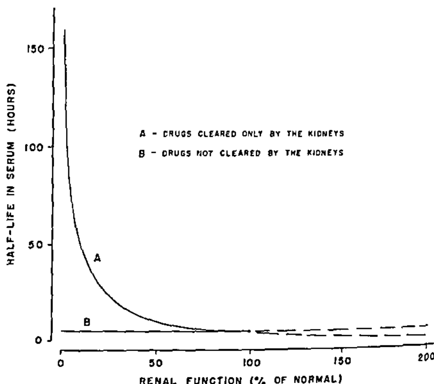


FIGURE 1 Representation of hypothetical relationship between half-life of drugs in serum and renal function for two classes of drugs

biotics in serum Two hypothetical drugs are considered drug A, which is completely stable and cleared only by renal mechanisms, and drug B, which is cleared entirely by nonrenal mechanisms An arbitrary half-life in serum for individuals with normal renal function is established at 5 hours for each drug in this model Additional factors which are considered in this model are presented elsewhere.[8]

A reciprocal relation between renal function and half-life in serum is found for drug A Note that a marked prolongation of serum half-life does not occur until renal function has fallen below 20 to 30 per cent of normal, at which point the blood urea nitrogen and creatinine will also rise. Curve B, on the other hand, reflects the fact that the serum half-

life of drug B is unaffected by the extent of renal dysfunction or hyperfunction

Two relatively simple methods can be used to determine whether or not a drug tends to resemble the hypothetical drugs A or B In general, drugs which, following parenteral injection, are found in large amounts in the urine resemble drug A, and those in which urinary recoveries are low tend to resemble drug B We have found it helpful to compare the half-life in serum of a given drug administered by a single parenteral injection to an anuric or severely uremic patient with that observed in normal patients[4,6] By this approach it has been possible to quantitate the effect of renal failure on the persistence of each antibiotic in the blood

Although drugs such as tetracycline, streptomycin, kanamycin, chloramphenicol metabolites, and others, have been found to have characteristics in common with drug A, they are excreted or inactivated in part by nonrenal mechanisms as well Although active chloramphenicol and to a lesser extent erythromycin and penicillin resemble drug B in many respects, all of these drugs are excreted into the urine to varying extents It has therefore been necessary to construct empiric curves which delineate the relation between the half-life in serum and renal function

In the case of the antibiotics most dosage schedules have been derived empirically and provide for a large excess of drug beyond that necessary for maximum effectiveness Such wide latitude is desirable in the case of nonuremic patients, but in uremic patients given potentially toxic drugs more care in dosage seems indicated

The level of drug in the blood immediately following a single intravenous injection depends upon the relative volume of distribution and the weight of the patient and therefore can be calculated Values for the relative volume of distribution of streptomycin have been reported by Boxer *et al*,[3] of a number of different antibiotics by Spitzy and Hitzenerger,[8] and of the tetracyclines by Kunin, Dornbush, and Finland[7] The ersistence of each drug in the blood depends upon the elimination rate nstant This function may be calculated from the half-life data presented Table I Methods for calculation of dosage are presented by Boxer *al*,[3,4] It is evident from examination of Figure 1 and of empirical curves structed for drugs resembling drug A that the elimination rate conts of each antibiotic may vary depending upon the severity of renal tional impairment

ONTROL OF THE CONCENTRATION OF ANTIBIOTICS IN THE URINE

concentration of an antibiotic excreted into the urine at any point depends upon the concentration in the blood, the rate at which it

is cleared by the kidneys, the rate of urine flow, and the functional capacity of the kidneys These relationships are set forth in the following equation:

$$(1) \qquad \frac{Ua}{Pa} = \frac{Cla{:}GFR}{V}$$

Where Ua/Pa is the ratio of urine to plasma concentration, V is the rate of urine flow in milliliters per minute, GFR is the glomerular filtration rate in milliliters per minute, and Cla is the ratio of the clearance of the antibiotic to the substance used to measure the GFR (usually endogenous creatinine).

This formula is used to construct the theoretical curves presented in Figure 2. Three hypothetical antibiotics are considered in this figure, each is identical with the others in all respects except the ratio of the

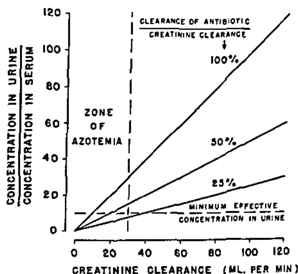


FIGURE 2. Relation between urine/serum concentration ratios and creatinine clearance for three hypothetical antibiotics which have renal clearances that are 100 per cent, 50 per cent, and 25 per cent, respectively, of the creatinine clearance.

rate of renal clearance to that of creatinine. One drug is cleared at a rate equal to that of creatinine (100 per cent), a second is cleared half as well (50 per cent), and the third one-quarter as well (25 per cent) The rate of urine flow is 1 ml. per minute. The horizontal broken line roughly delineates the minimum effective concentration ratio of drug in the urine, and the vertical broken line marks the level of creatinine clearance below which azotemia begins to appear.

This figure indicates that when the concentration of an antibiotic in the blood and rate of urine flow are constant, its concentration in the urine will be directly proportional to its renal clearance and to the renal functional capacity. Thus, for the treatment of infections of the urinary tract, high concentrations of drug in the urine will be more readily achieved by rapidly excreted antibiotics, even in the presence of uremia. Conversely, antibiotics that are slowly cleared may not reach effective concentrations even in patients with fair renal function unless the serum level is considerably elevated. These considerations may enter into the choice of drug, as, for example, the most favorable tetracycline analogue, *for the treatment of urinary tract infections (see Table I), all other* factors being equal

A detailed review of these problems for the antibiotics in current use has been published.[8]

Summary and Conclusions

Relatively simple pharmacologic principles have been applied to the problem of the restrictions that may be imposed by renal failure on antibiotic therapy.

The results of determinations of blood levels of antibiotics may serve as a useful guide to the need for such restrictions The size and frequency of doses which may properly be used in patients with impaired renal function depend largely upon the elimination rate constant and the apparent volume of distribution of the drugs

For drugs that are excreted largely by the kidneys, the elimination rate constant of an antibiotic is decreased and the related "half-life" of the antibiotic in the blood is markedly prolonged in patients with renal failure. The exact change in these rate measurements that is produced by any given antibiotic as a result of renal failure can be estimated, but is best determined empirically for each drug

In general, the more rapidly a drug is cleared by the kidney, the more likely it is to appear in the urine at therapeutic levels in spite of renal failure when the levels in the blood are at the usually desired concentrations.

Some of the factors which influence the limitations imposed by renal failure are summarized in Table I.

It is hoped that the principles and data reviewed here will serve as useful guides to antibiotic therapy in patients with anuria or with severe but lesser degrees of renal functional impairment.

ACKNOWLEDGMENTS

The author is indebted to Dr. Maxwell Finland, under whose guidance the concepts outlined in this paper were developed, and to the Editors of the *Journal of Clinical Investigation* and the *A.M.A. Archives of Internal Medicine* for permission to republish Figures 1 and 2, respectively.

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*The Nephrotoxicity of Antibiotics: A Review**

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Antibiotics have become indispensable in the therapy of urinary tract infections. As their use and abuse become more extensive, the possible toxicity of these agents must be critically evaluated This report represents a review, primarily of the English-speaking literature, of the nephrotoxicity of the antibiotics listed in Table I Renal hypersensitivity reactions to these antibiotics have been omitted

TABLE I NEPHROTOXICITY OF ANTIBIOTICS *

Name	Animals	Humans
Penicillin	0	0
Tetracyclines	0	0
Chloramphenicol	+?	0
Erythromycin	0	0
Streptomycin	0	+
Viomycin	0	0
Vancomycin	0	0
Neomycin	+++	+
Kanamycin	++	++
Bacitracin	+++	+++
Polymyxin A and D	+++	+++
Polymyxin B	+++	++
Novobiocin	+++	0

*0 = No nephrotoxicity, + = irritative, ++ = moderate toxicity, +++ = severe toxicity

Both human and animal studies have been evaluated for the following (1) the presence or absence of nephrotoxicity, (2) species differences, (3) the dose necessary to produce alterations in renal structure and/or

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function, (4) relationship of the nephrotoxic dose in animals to the maximal therapeutic dose in humans, (5) localization of the pathologic changes in the nephron, (6) the type and adequacy of the measurements of renal function, (7) mechanisms responsible for the nephrotoxicity, (8) the authors' over-all evaluation of the data.

Antibiotics Without Nephrotoxic Properties

These are listed in Table I, from penicillin through vancomycin (References 1, 2, 16, 19, 22, 24, 26, 29, 30, 32, 33, 42, 44, 46, 47, 49, 55, 58, 60, 71, 72, 78, 79, 80, 82, 84). The references listed are those in which the presence or absence of nephrotoxicity was specifically noted. One report (21) ascribed renal toxicity to the tetracyclines and chloramphenicol in rabbits at doses of 150 to 250 mg. per kilogram of body weight per day This study was at variance with those performed on mice, rats, cats, and dogs at comparable dose levels in which nephrotoxicity was not observed (29, 30, 38, 47, 58, 60, 82). The rabbits were extremely toxic, with prominent symptoms of anorexia, diarrhea, convulsions, and respiratory failure. Pathologic examination showed marked tubular degeneration, cellular and protein casts, and irregular crystals, resembling cystine crystals, in the tubular lumens. The liver showed lobular necrosis. It was not possible to exclude prerenal factors, such as fluid and electrolyte loss and shock, as the cause of the renal tubular degeneration in this study.

Although the earliest lots of streptomycin commercially available caused questionable renal damage, purification of the drug seems to have abolished this side effect (79). Possibly 20 per cent of humans receiving large doses of streptomycin (40 to 50 mg. per kilogram per day) may demonstrate mild transient albuminuria and cylindruria. Abnormalities in renal function have not been observed when streptomycin is administered to subjects with normal renal function (19, 22, 33, 49, 79, 84). Previous renal damage may predispose the individual to a nephrotoxic effect of streptomycin (19, 33, 49, 79). However, in these cases it is extremely difficult to rule out prerenal factors rather than streptomycin as the cause of the increased azotemia (5, 16, 19, 44, 52, 84).

Antibiotics with Specific Nephrotoxic Properties in Animals or Humans

These are listed in Table I and their effects are summarized in Tables II and III.

TABLE II. NEPHROTOXICITY OF ANTIBIOTICS — ANIMALS *

	Species	Dose (mg/Kg./day)	Function	Pathology	Comments
Neomycin	Rats, guinea pigs, cats, dogs	25–100	0	Proximal tubular necrosis >30 mg/Kg/day †	2 to 10 times maximal human dose ‡
Kanamycin	Mice, rats, cats, dogs	50–400	N < 100 mg/Kg.	Proximal tubular necrosis >200 mg/Kg	2 to 16 times maximal human dose
Bacitracin	All	6000–300,000 units	?N (NPN)	Proximal tubular eosinophilic degeneration to necrosis	(1) Tubular necrosis noted in mice only (2) 2 to 100 times maximal human dose
Polymyxin A and D Polymyxin B	Mice, rats, dogs All	2.5–40 2.5–20	0 2.5 mg N 5.0 mg GFR, [illegible] RPF [illegible]	Tubular necrosis Tubular cloudy swelling >20 mg/Kg	Not in clinical use (1) Distinct functional pathologic disparity (2) Possibly toxic at maximal human dose
Novobiocin	Dogs	500	(PSP)	Severe degeneration of kidneys	(1) Single experimental study (2) 20 times maximal human dose

* 0 = No data N = normal or no change, ? = inadequate data
† The dose at which tubular necrosis is noted
‡ The extent to which the dose administered to animals exceeds the maximal therapeutic dose
§ R = reversible alteration in function

TABLE III NEPHROTOXICITY OF ANTIBIOTICS – HUMANS *

		Function				
	Dose (mg/Kg)	NPN ↑	GFR	RPF	Urea Clearance	Tubular Function
Neomycin	15–40	Approx 15% ⇅	0	0	N	PSP N
Kanamycin	15–80	15% ⇅	50% ⇵	35% ⇵		35% ⇵
Bacitracin	500–4000 units	30% ⇅	100% ⇵	100% ⇵		100% ⇵
Polymyxin A and D	1 0	?	?	?	?	?
Polymyxin B	2 5–7 0	5% ⇅	N inulin	100% ⇵ PAH clearance		100% ⇵ T_mPAH
Streptomycin	10–40	N	?	?		N

* O = no data, N = normal or no change, ? = inadequate data
† The dose at which tubular necrosis is noted
‡ No significant toxicity at doses below this level

Neomycin

Animals Moderate to severe renal tubular damage has been observed in rats, guinea pigs, cats, and dogs at dose levels of 25 to 100 mg per kilogram per day (40, 59, 60, 77); this is two to ten times the recommended therapeutic dose in humans The greater the dose, the more marked the renal pathology. The histologic changes have been strikingly consistent. Necrosis is confined to the cells of the proximal convoluted tubules In those animals that survive, regeneration of the tubular epithelium is observed. Glomerular structure is not affected Although the animals die in uremia, no renal hemodynamic or tubular function studies have been carried out. Neomycin appears to exhibit minimal nephrotoxicity in animals in doses less than 20 mg. per kilogram per day. The mechanisms responsible for the renal toxicity of neomycin are unknown and have not been investigated The partial protection afforded by the simultaneous administration of pantothenic acid suggests coenzyme A as a possible site of action (41).

Humans (15, 18, 50, 61, 62, 76, 77). As might be expected, the data for humans are far less reliable than those in the animal studies. Many of the patients had pre-existing renal disease In others, it was often impossible

Sediment			Pathology	Comments
Albumin	Casts	RBC		
>60% ↑	>75% ↑	N	Proximal tubular necrosis at >40 mg/Kg †	N<20 mg/Kg †
15% ↑	10% ↑	10% ↑	?	N<20 mg/Kg
25-100% ↑	?	N	Necrosis of all proximal tubules (1 case)	Renal glycosuria in 7%
?	?	?	?	
30-50% ↑	?	5% ↑	?	(1) N<2.5 mg/Kg/day (2) Species differences
20% ↑	50% ↑	N	?	(1) Irritative only (2) Toxic with intrinsic renal disease

to determine whether the pathologic changes were due to the direct effect of neomycin on the kidney, the effect of another drug simultaneously administered, or the effect of the underlying illness and its complications. The most extensive study of the nephrotoxicity was that by Waisbren and Spink (76). This was a report of the treatment of various types of infections in 66 humans ranging in age from 9 months to 93 years. Thirty-six of the patients had albuminuria prior to therapy with neomycin. It is impossible to tell the incidence of increasing or initial albuminuria in the entire group from the published data, although the authors state that 6 of 9 patients who did not have prior albuminuria developed a trace to 1 plus during therapy. Twenty-four of 32 patients transiently developed fine granular casts. The authors do not state whether any of these 24 subjects had prior albuminuria. Thirty patients had serial determinations of blood urea nitrogen. Only 5 had temporary increases greater than 5 mg per cent above the control level. All 5 had prior renal damage. PSP tests and urea clearances done before, during, and after therapy did not show any significant or constant deviation. The authors do not indicate how many patients underwent these function tests.

Pathologic examinations of the kidneys were available in 11 patients who died during or shortly after completion of a course of neomycin therapy (15, 50, 61, 62, 76); 7 had renal pathologic changes ascribed to neomycin toxicity (15, 50, 61, 62, 76). A more careful analysis of these 7 cases is as follows

(1) An adult with bacterial endocarditis received 2 Gm of neomycin daily for 19 days. He had abnormal urinary sediment findings prior to therapy. The patient died 3.5 months after completion of neomycin therapy. He had severe congestive failure with anasarca, and he was given multiple diuretics without success, the type of diuretic unspecified.

Autopsy The collecting tubules and loops of Henle showed vascular nephropathy. The glomeruli were normal (62).

(2) A 55-year-old male had a perforated appendix with severe generalized peritonitis After surgery, 3 Gm. of neomycin (1 per cent solution) were given intraperitoneally. At no time was the patient hypotensive and no transfusion reaction was observed, BUN was normal preoperatively. Parenteral penicillin and streptomycin were also given. Progressive renal failure began 24 hours after neomycin administration. No oliguria was present, but BUN rose to 200 and the patient expired.

Autopsy showed swelling and hydropic degeneration of the proximal tubule with areas of epithelial regeneration (50).

(3) Neomycin, 0.5 Gm. per day, and streptomycin, 1 Gm. per day, were given for 26 to 39 days to three children, ages 2, 2, and 4 years with tuberculous meningitis. Weights were 12, 12, and 13 Kg., respectively. The last week of therapy they developed "heavy" proteinuria, and in one the NPN reached 39 mg. per cent In the other two, the NPN was normal.

Autopsy All three showed necrosis, vacuolation, and sloughing of the cells of the proximal convoluted tubules. Glomeruli were normal (61).

(4) An adult was treated for tubulerculosis with 2 Gm of neomycin daily for somewhere between 13 and 30 days. The patient showed evidence of "renal impairment" but *died of his underlying disease*

Autopsy: Extensive necrosis and sloughing of tubular epithelium with incomplete regeneration were shown All segments of the nephron were involved Tubules were filled with eosinophilic casts Glomeruli appeared normal (15)

(5) A 42-year-old patient died with acute renal shutdown while receiving neomycin, promizole, streptomycin, and sulfadiazine.

Autopsy "Severe toxic tubular damage was evident" (76)

These cases illustrate the difficulty of attributing the renal pathologic changes to neomycin per se. It might be concluded that number 2 and number 3 (3 cases) represented nephrotoxicity to neomycin. The sites of the lesions resembled those in animals studied, and the size of the dose administered was excessive. A systematic study of renal hemodynamics and tubular functions was not reported in any of the investigations in humans on the clinical use and toxicity of neomycin.

Kanamycin

Animals Nephrotoxicity has been investigated in mice, rats, cats, and dogs. The available data suggest that in these animals kanamycin is significantly less toxic, on a weight basis, than is neomycin (70) Dogs, rats and cats demonstrated no nephrotoxicity histologically or functionally when this antibiotic was administered for intervals of 40 days to 9 months at doses up to 100 mg. per kilogram per day. At a dosage

range of 100 to 200 mg per kilogram per day, cloudy swelling of the proximal convoluted tubules and minimal tubular necrosis occurred despite the absence of clinical signs of toxicity. With doses in excess of 200 mg. per kilogram per day, albuminuria, hematuria, and azotemia developed. Anuria followed if the drug was not discontinued. In the latter animals, severe proximal and moderate distal tubular necrosis was observed without significant glomerular changes.

Humans. In contrast to the animal data, the human studies demonstrated evidence of nephrotoxicity by renal functional studies at 25 to 50 mg. per kilogram per day (7, 9, 14, 17, 23, 81). This of course suggests a rather striking species difference. In one investigation, renal impairment of varying magnitude was noted during and after treatment with kanamycin in 100 per cent of 20 men who received doses between 25 and 50 mg. per kilogram per day. In all of these patients, renal function was normal prior to therapy. The average duration of therapy was 20.2 days. Renal function was evaluated by the following tests: inulin, creatinine, and para-aminohippurate clearances, serum creatinine, PSP excretion; maximal concentration and dilution tests. Routine urinalyses were done. Creatinine and inulin clearances were significantly reduced in approximately 50 per cent and PAH clearances in 35.2 per cent. Thirty-eight per cent exhibited a reduction in maximal urinary concentration. Proteinuria appeared in 10 per cent and microscopic hematuria in 20 per cent, cylindruria was not observed. Every patient showed impairment in at least one major function, but parallel qualitative and quantitative changes were not observed for all functions in any individual. This finding emphasizes the need for measurement of many specific functions in evaluating the nephrotoxic effect of any antibiotic.

In three other studies, 156 patients received kanamycin for the treatment of a large variety of infections. Urinalyses, blood urea nitrogens, and/or creatinines were measured in all cases. Mild reversible abnormalities in renal function were observed in approximately 10 to 20 per cent. When careful follow-up observations were available, demonstrable abnormalities returned to normal within 2 months. Unfortunately, no reliable histologic observations of the kidneys of the above patients were reported. A percutaneous renal biopsy was done on one patient by Berman and Katz (8). This patient was severely toxic from a right upper lobe pneumonia. Treatment with 25 mg. per kilogram per day of kanamycin was given for 13 days. Prior to therapy the BUN was 31 mg per cent and the urine contained protein and polymorphonuclear leukocytes. On the thirteenth day of therapy, the patient became oliguric and had a recurrence of fever, and the BUN rose to 75 mg. per cent. The renal biopsy done 25 days after onset of oliguria showed healing

There is suggestive evidence that the nephrotoxic effect of bacitracin is due to its polypeptide structure. Very similar lesions may be produced in animals by the administration of polypeptides and certain amino acids The most striking changes have followed the acute or chronic administration of dl-serine. Simultaneous ingestion of methionine, glycine, or alanine afforded striking protection against the nephrotoxic effect of dl-serine. The most likely cause of the nephrotoxicity of this amino acid is the tubular contact or tubular reabsorption of the unmetabolized D isomer (74, 75). The protection afforded by the other amino acids is probably due to the competitive inhibition of the tubular reabsorption of dl-serine. Unfortunately, no studies are available on the protective effects of amino acids against the nephrotoxicity of bacitracin.

Polymyxins

These polypeptide antibiotics appear to have nephrotoxic properties comparable to those of bacitracin, although the functional studies in humans are far less complete. The polymyxins A, B, C, D, and E comprise a group of closely related polypeptides They have been shown to have marked differences in their nephrotoxicity (6, 11, 13, 35, 56, 63, 68) All but polymyxin B (Aerosporin) have been discarded for clinical use because of their toxic properties.

Animals. In all animals, polymyxin A and D produced severe necrosis of tubular epithelium (primarily proximal) and marked abnormalities in the urinary sediment (6, 11, 13, 35, 56, 63, 68). The subsequent discussion will be confined to polymyxin B.

Thirty-one dogs receiving 2.5 mg. per kilogram per day for 4 to 6 weeks (56) had no alteration in renal function when measured by creatinine and PAH clearance and T_m glucose. At 5.0 mg per kilogram per day, 70 per cent of the animals showed moderate to severe reduction in creatinine clearance and glucose T_m, but PAH clearance remained normal No examinations of the urinary sediment were done. Those dogs sacrificed during treatment showed only "cloudy swelling" of the tubules In another study (11), dogs, rats, and rabbits receiving 12 mg per kilogram per day for 4 days showed no sediment or pathologic changes Rats and dogs receiving 200 mg per kilogram per day for 4 days and sacrificed on the fifth day displayed minimal tubular degeneration. The functional-pathologic dissociation is rather striking in these animal studies Only minimal pathologic changes were observed at 20 mg. per kilogram per day, but significant functional alterations occurred at 5 mg per kilogram per day (56). The latter is a dose level commonly used in clinical studies

Humans. T_m PAH and inulin clearance were measured in 10 patients before and after receiving 2.5 mg of polymyxin B per kilogram per day for 2 weeks (83). T_m PAH fell approximately 10 per cent in all cases

(questionable significance) without a change in inulin clearance. Four additional patients with mild prior renal impairment showed marked reduction in inulin clearance and T_m PAH, with only partial subsequent recovery. No other investigations had specific renal function studies. The incidence of urinary sediment abnormalities and increased NPN are listed in Table III (32, 39, 63, 64). The available data would suggest that polymyxin B is not nephrotoxic at doses of 2.5 mg per kilogram per day or less when administered to individuals with normal kidneys.

It has been demonstrated that substances which protect against the nephrotoxic effects of dl-serine in animals also protect against the nephrotoxic effect of the polypeptide polymyxin (11, 68). dl-Methionine was most protective in animals but only partially protective in humans. It is possible that the other major polypeptide antibiotic, bacitracin, produces nephrotoxicity in a manner comparable to polymyxin.

Novobiocin

Although nephrotoxicity has been observed in dogs receiving 300 mg per kilogram per day of novobiocin for 60 days (45), no nephrotoxicity has been reported in humans (25, 27).

CONCLUSION

This review in general points up the inadequacy of the available studies on the nephrotoxicity of antibiotics. With the exception of the mechanism suggested for the renal toxicity of the polypeptides, polymyxin and bacitracin, no data are available with reference to the other antibiotics studied. Nephrotoxicity seems to be correlated with the blood and/or urine levels of the active antibiotic. As a corollary, the duration of administration or total dose seems much less important than the daily dose in milligrams or units per kilogram.

With regard to the problem of the renal toxicity of antibiotics, certain general statements seem warranted. (1) Nephrotoxicity reported in animal studies generally was noted at doses considerably in excess of the maximal therapeutic levels in humans. (2) Systematic studies of individual renal functions and renal histology are not available for most antibiotics. (3) Most clinical investigations of the renal effects of the antibiotics are complicated by possible renal damage from the underlying disease for which the antibiotic is administered. (4) Critical animal studies on chemical structure and nephrotoxic interrelationships are not available. Furthermore, we were unable to find a single investigation on animals where pair-fed animals were also studied. Frequently toxic doses of an antibiotic produced appetite alterations, nausea, vomiting, and hy

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*Studies on the Pathology and Therapy of Experimental Enterococcal Pyelonephritis in Mice**

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This report will present our observations on the nature and control of a chronic experimental enterococcal infection in mice. That this disease proved to be hematogenous pyelonephritis was fortuitous and secondary to our objective of developing a reliable and useful mouse infection with *Streptococcus faecalis*. It must be pointed out, as for most experimental infections, that our findings may permit only a limited extrapolation to human disease. This limitation might apply particularly to the route of infection, both for our experiments with mice and for those recently reported with rats,[2] since these animal infections were hematogenous and the clinical data from human enterococcal disease suggest that it is urogenous in most cases.[3]

Despite the fact that enterococci have long been recognized as human pathogens, especially in urinary tract infections,[8, 11, 13, 14] an experimental enterococcal infection had not been described when we began our studies. Indeed, it was concluded a good number of years ago that enterococci were practically avirulent for mice and rabbits.[5] We felt that the matter needed reinvestigation, and accordingly screened a number of recently isolated strains of enterococci for mouse pathogenicity. These strains came from patients with urinary tract infections, and all strains proved capable of persistent localization in the mouse kidneys after intravenous challenge. However, one strain (designated as "MGH-2" and identified as *Streptococcus faecalis* var. *zymogenes*) appeared to be especially consistent as a mouse pathogen, and the experiments to be described were performed with this strain.

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Basic Bactfriologic Aspects

When 15 to 17 Gm male CF-1 mice in groups of 20 were injected intravenously with varying numbers of enterococci and sacrificed 7 days later, the results of kidney cultures from each group revealed a linear relationship between the logarithm of the number of cocci injected and

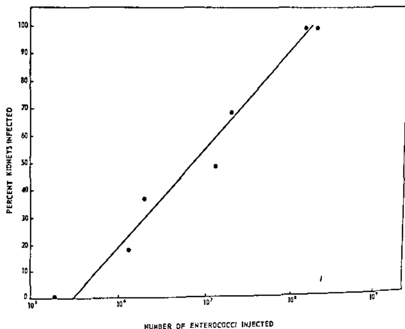


Figure 1. Relationship between size of challenge dose of *Streptococcus faecalis* and incidence of infected mouse kidneys.

the incidence of culturally positive kidneys. This relationship is depicted in Figure 1, wherein the minimum infective dose was about 10^5 cocci and the 50 per cent infective dose was 8×10^6 cocci Parenthetically, the enterococcal 50 per cent infective dose approximated that reported for a staphylococcal pyelonephritis in mice[6] In all of the experiments to follow, we employed a challenge dose of about 10^8 enterococci, which is the 90 to 100 per cent infective dose.

The bacteriologic course of the infection induced with 10^8 cocci is illustrated in Figure 2. Groups of 10 mice were sacrificed at intervals throughout 152 days, and their organs and blood samples were homogenized, pooled, and subjected to plate count evaluations. It should be

emphasized that the data in Figure 2 are derived from pooled group specimens, a procedure which indicates only the status of the group and does not reveal any information on variation between individual mice. One pole of each kidney (approximately one-third kidney) was used for bacteriologic studies, and the remainder was utilized for histopathologic evaluation. To recapitulate from our previous report,[4] it was

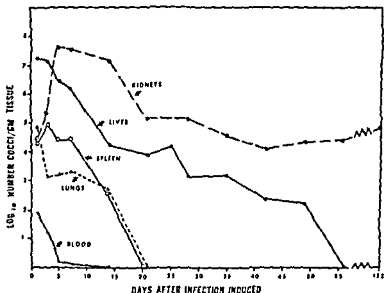


FIGURE 2 Enterococcal populations of the blood and various organs of mice sampled over a 152-day period subsequent to intravenous challenge.

evident that whereas the cocci were at first systemically seeded in large numbers, they were gradually eliminated from all of the organs examined except the kidneys. The renal count indicated a rapid growth phase of about 7 days, followed by a decline to a more or less stabilized level of 10^4 to 10^5 cocci per gram, maintained for nearly 20 weeks through the end of the experiment. It is noteworthy that the mortality was only 5 per cent among the mice observed for 152 days, which is in contrast to the high mortality which occurs in analogous staphylococcal infections in the same species.[4, 5, 17]

Basic Pathologic Aspects

The pathologic course of the infection was studied by gross and microscopic examination of the remainder of each of the kidneys from

Basic Bacteriologic Aspects

When 15 to 17 Gm. male CF-1 mice in groups of 20 were injected intravenously with varying numbers of enterococci and sacrificed 7 days later, the results of kidney cultures from each group revealed a linear relationship between the logarithm of the number of cocci injected and

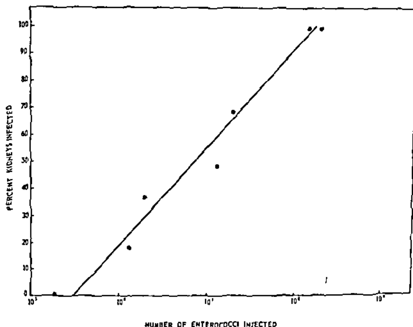


Figure 1. Relationship between size of challenge dose of *Streptococcus faecalis* and incidence of infected mouse kidneys

the incidence of culturally positive kidneys. This relationship is depicted in Figure 1, wherein the minimum infective dose was about 10^5 cocci and the 50 per cent infective dose was 8×10^6 cocci. Parenthetically, the enterococcal 50 per cent infective dose approximated that reported for a staphylococcal pyelonephritis in mice.[6] In all of the experiments to follow, we employed a challenge dose of about 10^9 enterococci, which is the 90 to 100 per cent infective dose.

The bacteriologic course of the infection induced with 10^9 cocci is illustrated in Figure 2. Groups of 10 mice were sacrificed at intervals throughout 152 days, and their organs and blood samples were homogenized, pooled, and subjected to plate count evaluations. It should be

emphasized that the data in Figure 2 are derived from pooled group specimens, a procedure which indicates only the status of the group and does not reveal any information on variation between individual mice. One pole of each kidney (approximately one-third kidney) was used for bacteriologic studies, and the remainder was utilized for histopathologic evaluation. To recapitulate from our previous report,[4] it was

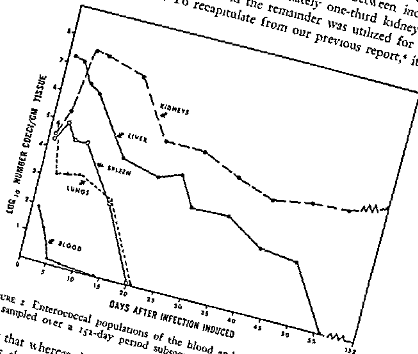


FIGURE 2 Enterococcal populations of the blood and various organs of mice sampled over a 152-day period subsequent to intravenous challenge

evident that whereas the cocci were at first systemically seeded in large numbers, they were gradually eliminated from all of the organs examined except the kidneys The renal count indicated a rapid growth phase of about 7 days, followed by a decline to a more or less stabilized level of 10^4 to 10^5 cocci per gram, maintained for nearly 20 weeks through the end of the experiment It is noteworthy that the mortality was only 5 per cent among the mice observed for 152 days, which is in contrast to the high mortality which occurs in analogous staphylococcal infections in the same species [4, 7, 21]

Basic Pathologic Aspects

The pathologic course of the infection was studied by gross and microscopic examination of the remainder of each of the kidneys from

all animals upon which the results in Figure 2 are based In addition, 5 groups of 10 animals each were sacrificed at intervals of 24, 32, 48, 56, and 72 hours post-challenge. A total of approximately 480 kidneys were studied All kidneys were examined microscopically in cross and coronal section with hematoxylin-eosin, PAS, trichrome, and Gram stains Serial sectioning was not undertaken.

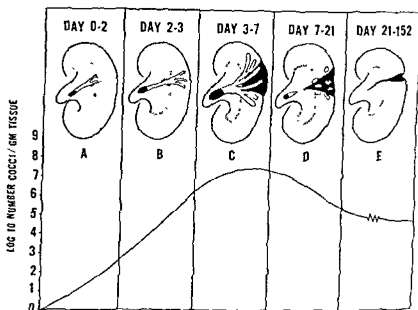


FIGURE 3. Sequence of major histopathologic changes in relationship to bacterial population in kidneys. In black, infiltrative lesions, dotted areas, dilated tubules.

The gross pathology encountered in the kidneys was of a subtle nature in contrast to the obvious large multiple abscesses produced in mouse kidneys by staphylococci[6, 7, 17] Only occasionally were focal surface areas of pallor evident. These areas tended to be mildly elevated during the early stages of the infection and small and slightly depressed in later sacrifice groups. In general the kidneys remained within normal size limits on gross inspection.

The pathologic course of the renal infection as revealed by microscopic study is illustrated diagrammatically and photomicrographically in Figures 3 to 6 It should be emphasized that the diagrams and photomicrographs have been selected to demonstrate the sequence of major pathologic alterations which were encountered at the respective time intervals, and do not present the details of pathogenesis.

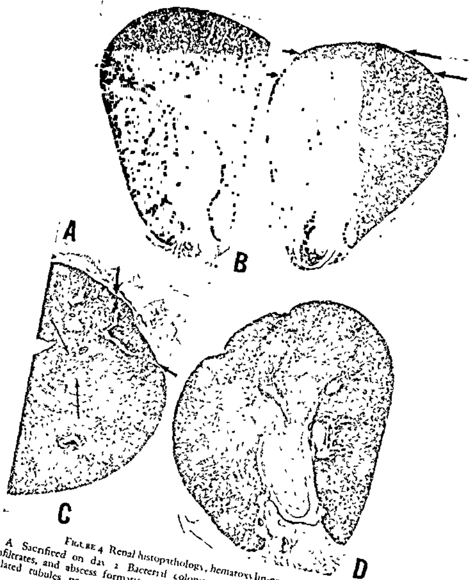


FIGURE 4 Renal histopathology, hematoxylin-eosin

A Sacrificed on day 2 Bacterial colonies, polymorphonuclear leukocytic infiltrates, and abscess formation in papilla Note (arrows, circular pattern) dilated tubules near corticomedullary junction Arrow in papilla denotes bacterial colony (also see Figure 5A) Magnification ×15

B Sacrificed on day 7 Necrotizing papillitis with extensive retrograde intrarenal hydronephrosis of entire kidney and superimposed infiltrative lesions (arrows) Magnification ×10

C Sacrificed on day 21 Wedge-shaped infiltrative lesion with mildly depressed base on cortical surface and apex extending into medulla (arrows) Note absence of significant tubular dilatation Magnification ×12

D Sacrificed on day 56 Narrow wedge-shaped lesion extending into papilla with two depressed scars on cortical surface Magnification ×11

The most prominent abnormality which was encountered at the end of the second day following bacterial challenge consisted of the appearance of bacterial colonies and acute polymorphonuclear leukocytic infiltrates in the medulla of the kidney (Figures 3A, 4A, and 5A). Some of the lesions had progressed to form small abscesses, which characteristically resulted in occlusion and retrograde dilatation of the papillary ducts passing through the involved area (Figures 3A and 4A). Occasional small acute infiltrative lesions were encountered in the base of the pyramid near the corticomedullary junction at this time Of the 10 animals sacrificed at the 2-day interval, 6 exhibited acute papillary lesions, accompanied by variable degrees of mild tubular dilatation extending to but not beyond the corticomedullary junction. None of the sections (20 kidneys, 60 sections) at this time interval exhibited any evidence of acute cortical infiltration.

During the third day (Figure 3B) the papillary abscesses had become more prominent, and the dilatation of the duct system had extended well into the cortex, where it was accompanied by areas of acute leukocytic infiltration. Such infiltrates were encountered in 60 to 70 per cent of the kidneys at this time, representing 90 to 95 per cent of the animals examined.

During the remainder of the first week (Figures 3C and 4B) the papillary lesions reached their maximum development. In some animals these lesions remained relatively small and were associated only with segmental retrograde dilatation and acute cortical infiltration. In other animals, however, the papillary lesions involved the entire tip of the papilla in the form of a necrotizing papillitis. Such lesions resulted in widespread and generalized intrarenal hydronephrosis, illustrated in Figure 4B. The generalized hydronephrosis, however, was not necessarily accompanied by generalized cortical infiltration. Thus the typical wedge-shaped infiltrative lesions of pyelonephritis became superimposed upon the general hydronephrotic tubular dilatation, as shown in Figure 4B While the early (24 to 56 hours) stages of dilatation appeared to involve only the papillary, collecting, and distal convoluted segments of the nephrons, later progression resulted in similar dilatation of the proximal segments of the nephron as well This was particularly true in those cortical areas which were adjacent to but not involved in the infiltration process In contrast, the proximal tubular segments located within the wedge-shaped pyelonephritic lesions often appeared to be "afloat" amidst the infiltrative cellular elements, where they exhibited atrophic changes without dilatation. The fate of the proximal segments within the infiltrated areas, combined with evidence of a degree of glomerular degeneration and atrophy, suggests the possibility of vascular occlusion to some of the nephrons within the acute pyelonephritic lesion.

During the period from 7 to 14 days the papillary lesions underwent a significant regression and assumed a less acute appearance histologically (Figures 3D, 4C, and 5B). Concurrently the degree of both generalized and segmental internal hydronephrosis diminished, as a result of which only minimal tubular dilatation remained in many of the kidneys on the fourteenth day. Where present, the dilatation was limited in location, and occurred either within or adjacent to the infiltrated pyelonephritic areas (Figure 5B). Simultaneously, an increasing number of mononuclear cells (lymphocytes and plasma cells) became evident within the infiltrated areas (Figure 5B). As a result of these events, the surface of the pyelonephritic lesions became slightly depressed (Figures 3D and 4C).

Subsequent to the twenty-first day both the papillary and cortical lesions in general continued to undergo further regression in most of the animals. Evidence of prior papillary involvement in some of the animals consisted only of an area of scarring or mild papillary duct dilatation. In a small percentage of the animals, however, the architecture of the papilla was grossly disturbed, with the entire papilla consisting only of scar tissue containing several bizarre ducts of varying size. Such kidneys characteristically were small, atrophic, heavily scarred throughout, and possessed relatively little normal renal tissue.

Subsequent to the twenty-first day the cortical lesions became progressively smaller in size (Figures 3F and 4D) and were noted on the surface of the kidney as small contracted scars. If suitably sectioned, such lesions could be traced continuously into the area of residual scarring in the papilla. As progressive healing occurred, many of the cells in the cortical lesions assumed a fibrotic appearance (Figures 6A and 6B).

Although the series of events depicted above has emphasized the development and regression of the lesions encountered, it should be pointed out that small foci of acute activity, taking the form of a microscopic accumulation of polymorphonuclear leukocytes in the interstitial tissue or as part of an occasional cellular cast, could be identified upon careful examination. Such evidence was minimal, however, and it is worthy of emphasis that the over-all appearance of the lesions after the twenty-eighth day was one of inactivity, chronicity, and healing. This finding was encountered in spite of the fact that approximately 90 per cent of kidneys may be expected as late as 42 days to yield positive bacteriologic cultures (Table II).

The tendency for intravenously administered enterococci to localize initially in the medulla of the mouse kidney is similar to results obtained in this species[10] and in the rabbit[7] with *Corynebacterium renale*. With the latter bacteria, however, the animals succumb at a time when the necrotizing papillitis is the predominant lesion and prior to the establishment of the type of chronic pyelonephritic lesions encountered in the

present study. The initial medullary localization of enterococcal lesions is in contrast to the random medullary and cortical distribution of abscesses produced by the staphylococci in mice[6, 7, 17] and by *Escherichia coli* when administered to rats in conjunction with renal massage.[1] It should also be noted that the present findings in mice are dissimilar to those obtained with experimental enterococcal pyelonephritis in rats,[2] particularly with regard to the relative prominence of the papillary and cortical lesions. A possible explanation of the observed differences relates to a variation between these species in the sensitivity of their kidneys to enterococcal challenge. However, the influence of renal massage in the previously reported results in rats should be considered.

Finally, the general pattern of the progression and regression of the renal lesions in the present study can be correlated with the growth and decline of the enterococcal population in the kidneys, as depicted in Figure 3. Thus, the rapid increase in bacterial numbers during the first week was associated with the maximum rate of progression of the acute renal lesions Following this period, there was a decrease in bacterial numbers over a 2-week interval and the acute pyelonephritic lesions underwent a transformation into the chronic phase.

Antibacterial Treatment

Our initial studies were concentrated on combined penicillin-streptomycin treatment, since this combination has been recommended as the therapy of choice for enterococcal urinary tract infections in man[5, 9, 11] The MGH-2 strain was completely inhibited *in vitro* by penicillin G at 1.6 to 3 1 micrograms per milliliter and by streptomycin at 100 to 200 micrograms per milliliter, determined by broth-dilution tests. As shown in Figure 7, the bacteriostatic levels of 5 and 100 micrograms per milliliter of penicillin and streptomycin, respectively, caused a distinct synergistic bactericidal action on this enterococcus *in vitro*. These results almost exactly duplicate the experience of others[8] on the combined action of penicillin and streptomycin on an enterococcus Moreover, as will be shown later, synergism between these antibiotics was also observed in the mouse infection.

In order to elicit a high level of activity, our first experiments on penicillin-streptomycin treatment were essentially prophylactic. These two antibiotics were administered beginning on the day of challenge Groups of 20 mice were employed per regimen, and s[illegible] [illegible]ycin or penicillin G, separately and as a [illegible] were given as [illegible] [illegible]cutaneous dose once daily for 5 c[illegible] [illegible]ys Three [illegible] [illegible]e last antibiotic dose, all mice were [illegible] their kidne[illegible] [illegible]lually cultured

Our preferred criterion for expressing antienterococcal activity is the "per cent sterile kidneys," the incidence of kidneys from a given group which were negative on culture. We believe that this criterion is more practical and perhaps more meaningful than the plate count enumeration procedure values which have been used for evaluating drug activity against staphylococcal kidney infections in mice[12]. In this and subsequent experiments, the enterococci recovered from treated mice were

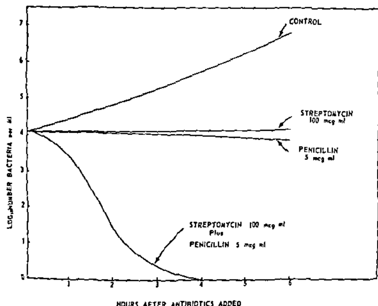


FIGURE 7. Bactericidal synergism between individually bacteriostatic levels of penicillin and streptomycin *in vitro*

routinely tested for penicillin and streptomycin sensitivity *in vitro*, and in no instance were we able to obtain any evidence of increased resistance to either antibiotic

The pooled results of several replicate experiments, wherein antibiotic treatment was started on the day of challenge, are given in Table I It is apparent that penicillin alone at 50 mg per kilogram per day or streptomycin alone at 100 mg per kilogram per day had only moderate activity When both agents *were given together* at these dosages, however, all of the kidneys were free of cultivable enterococci In short, the combined effect obtained here may be regarded as an instance of synergistic action *in vivo*, confirming the previously described observation *in vitro*, shown

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TABLE II Effect of Penicillin and Streptomycin on Enterococcal Pyelonephritis in Mice (Delayed Treatment)

Antibiotics and Dosage	Treatment Started Days Post infection	Total Days of Treatment	Per Cent Sterile Kidneys*	
			Untreated	Treated
Penicillin 50 mg /Kg /day plus Streptomycin 100 mg /Kg /day	0†	5	11	100
Penicillin 50 mg /Kg /day plus Streptomycin 100 mg /Kg /day	3	5	5	0
Penicillin 50 mg /Kg /day plus Streptomycin 100 mg /Kg /day	10	5	5	10
Penicillin 50 mg /Kg /day plus Streptomycin 100 mg /Kg /day	17	15	3	58
Penicillin 200 mg /Kg /day plus Streptomycin 100 mg /Kg /day	24	15	10	70

* 40 kidneys per regimen
† Data on this regimen taken from Table I

ineffective when commenced 3 or 10 days later Lacking further experimental evidence, we can only propose at this time that the discrepancy in the observed results between the two regimens is a reflection of the differences between the pathologic and bacteriologic states at each time period. The evidence, nevertheless, has established that the so-called synergism of a bactericidal antibiotic combination is apparent only under rather special conditions *in vivo*

Turning to the results obtained from the regimens started 17 and 24 days post-challenge, we may postulate that the increasing effectiveness of this treatment was perhaps due in part to the more intensive therapy, in part to lesser bacterial involvement, and in part to the partial recovery from renal damage which had occurred at this time Yet, notwithstanding the relatively massive antibiotic regimens, 30 to 42 per cent of the kidneys remained bacteriologically positive It may be of more than minor significance that, in additional experiments not described here, we found that approximately 40 per cent of kidneys from *untreated* mice taken 96 and 129 days after challenge were also positive on culture The question may be posed, therefore, whether a 40 per cent incidence of this experimental kidney infection in the later stages of the disease, whether treated or not, represents an irreducible minimum, in that the enterococci

in Figure 3. Interestingly, antagonism or interference was noted when penicillin and streptomycin combined treatment was applied for a staphylococcal pyelonephritis in mice.[11]

TABLE I Effect of Penicillin and Streptomycin on Enterococcal Pyelonephritis in Mice (Early Treatment)

Antibiotic	Dose* (mg /Kg /day)	Number of Kidneys	Per Cent Sterile Kidneys
None untreated	—	160	11
Penicillin G	50	80	31
Penicillin G	100	80	44
Penicillin G	200	160	86
Streptomycin	100	120	21
Streptomycin plus Penicillin	100 50	80	100

* 1 subcutaneous dose per day, 5 consecutive days, starting day of challenge

Since the aforementioned experiments involved early and brief treatment, it was of interest to study the effect of therapy started at various intervals after the infection was established, and when treatment was given for longer than 5 days In one such experiment, combined penicillin-streptomycin treatment was begun at 3, 10, 17, and 24 days after challenge, given for 5 consecutive days for one week or for 3 weeks, as indicated in Table II As before, single daily doses were administered subcutaneously The mice in each regimen, as well as untreated controls, were sacrificed 3 days after treatment was terminated, and each kidney was cultured. The results of this experiment are given in Table II.

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What we have found, in brief, is that a given antibiotic regimen was highly effective when instituted on the day of challenge and was totally

TABLE II EFFECT OF PENICILLIN AND STREPTOMYCIN ON ENTEROCOCCAL PYELONEPHRITIS IN MICE (DELAYED TREATMENT)

Antibiotics and Dosage	Treatment Started Days Post-infection	Total Days of Treatment	Per Cent Sterile Kidneys*	
			Untreated	Treated
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have acquired a relationship with the host which is beyond the effect of immunologic or chemotherapeutic factors. We may well be faced with the problem of "microbial persistence," a phenomenon which has not been satisfactorily explained and which has a great deal of clinical significance.[13] It is possible that these late stages of enterococcal pyelonephritis in mice may serve as an experimental model for studies of one kind of microbial persister.

A final experiment to be described represents a limited excursion into the area of immunology. A heat-killed (60° C. for 60 minutes) suspension of the MGH-2 enterococcus, containing about 10^9 cells per milliliter, was prepared. Two 10-mouse groups were injected subcutaneously with 0 2 ml. of this suspension, and these and additional groups were challenged 7 days later with 10^8 cocci intravenously. A vaccinated and nonvaccinated group were given penicillin G at 50 mg. per kilogram per day as a single subcutaneous dose once daily for 5 days, beginning on the day of challenge Untreated vaccinated and nonvaccinated control groups were also employed, and all mice were examined by kidney culture 7 days after the last penicillin dose. The results of this experiment are summarized in Table III

TABLE III EFFECT OF HOMOLOGOUS VACCINE AND PENICILLIN ON ENTEROCOCCAL PYELONEPHRITIS IN MICE

Regimen	Per Cent Sterile Kidneys*
None untreated	8
Penicillin 50 mg /Kg /day†	18
Vaccine‡	35
Vaccine plus penicillin§	45

* 20 kidneys per regimen
† 1 daily subcutaneous dose, 5 days, starting on day of challenge
‡ *1 subcutaneous dose given 7 days prior to challenge*
§ Dose of each agent same as given separately

Although this was a pilot experiment, the data given in Table III, as well s some additional experiments on vaccine immunity, have suggested hat specific antibacterial immunity may have some role in the control f this infection. We cannot draw any definite conclusions from the esults obtained thus far since the vaccine-induced immunity was somewhat limited and was only slightly effective as an adjunct to penicillin reatment.

Further assessment of the interplay of the pharmacologic, histopatho-)gic, bacteriologic, and immunologic elements of this experimental infec-

tion with respect to the effectiveness of antimicrobial drug treatment is contemplated, and it is our hope that the observations which can be made from this enterococcal infection may contribute toward a better understanding of the basic biology of pyelonephritis and of the therapy of this disease in man.

ACKNOWLEDGMENTS

The authors wish to thank Mr James L Schardein, Assistant Research Biologist, Parke, Davis and Company, for valuable technical assistance, the Art Department of Parke, Davis and Company for the preparation of illustrative material, and the Department of Medical Photography, Harper Hospital, Detroit, for the photomicrography.

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Principles in the Long-term Management of Chronic Infection of the Urinary Tract*

EDWARD H. KASS, M.D., and DONALD P. ZANGWILL, M.D.
(Boston, Massachusetts)

The treatment of chronic infections of the urinary tract is still in a most unsatisfactory state. A few years ago, when the subject was reviewed,[1] the conclusion was reached that the bacteriologic cure rate in chronic infections of the urinary tract was probably not more than 20 per cent despite the major advances in antibacterial therapy of the past two decades.

The reasons for the failure to achieve more satisfactory treatment in this group of infections are not clear. It is obvious that among the reasons for failure of treatment may be listed such factors as (*a*) the use of an ineffective drug, (*b*) the presence of substances or chemical circumstances that interfere with the antibacterial action of drugs, (*c*) the inaccessibility of the organisms to the action of the drug, either because they are not multiplying or because they are sequestered in a lesion, (*d*) the presence of mixed infections leading to the emergence after treatment of a previously suppressed strain of bacteria, (*e*) the implantation, spontaneously or by instrumentation, of new strains of bacteria into the urinary tract, (*f*) the occurrence of mutations within the urinary tract, with selection of resistant mutants during treatment, and (*g*) the failure of local antibacterial defense mechanisms to operate adequately.

It is not possible at present to assess the relative significance of these and other factors in the refractoriness to treatment of infections of the urinary tract. Yet it is necessary to adopt an operational approach to those patients who present with chronic infections of the urinary tract.

We wish to present our current view of the problem of management of these infections, and to indicate some of the underlying principles. No illusions are held that this is an ultimate form of therapy. Indeed, it would

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be depressing if this approach were not superseded within a few years

It is suggested that chronic infections of the urinary tract are maintained by persistent bacteriuria, and that the bacteriuria provides a focus for continuous reinfection of tissues of the urinary tract. It is assumed that the kidney and other tissues of the urinary tract tend to free themselves of infection spontaneously, unless continuously reinfected by the bacteria in the urine On the other hand it is conceivable and likely that foci of infection may persist in the tissues even after bacteriuria has been eliminated by appropriate treatment, because of interference with local defense mechanisms or because of unavailability of the microorganisms to the action of either antibacterial drugs or host mechanisms of defense.

Thus, it becomes essential that the urine be kept free of bacteria until the tissue foci have healed. The urine is kept free of bacteria by appropriate use of antibacterial agents and by avoidance of procedures that will introduce new bacteria into the urinary tract. With sufficiently prolonged suppression of bacteriuria, it is hoped that the tissue foci will become free of bacteria spontaneously, as they do in most experimental infections, even those associated with obstruction of the urinary tract *Antibacterial agents would thus act principally on the bacteriuria, but* would be needed at tissue levels to treat actively spreading infections or to treat those foci of infection that resist spontaneous healing. The length of time that suppression of the bacteriuria should be maintained in accordance with this hypothesis is unclear and arbitrary units of three to six months of treatment have been selected

The long-term suppression of bacteriuria is a key aspect of this approach. Many drugs have been used to achieve such suppression, and several long-term studies have suggested that prolonged treatment with an effective agent may be advantageous in the management of chronic infections of the urinary tract.[4, 6, 11] Sulfonamides have ben widely used, and are often effective. However, many patients with chronic infections of the urinary tract already have sulfonamide-resistant infections. Toxicity is occasionally a problem, and the fear of long-term consequences of the prolonged use of sulfonamides (polyarteritis nodosa, for example) is real Nevertheless, if bacteriuria can be successfully suppressed by sulfonamides, it is reasonable to continue their use for many months, in the hope that the infection will ultimately be eradicated. Similar considerations obtain for nitrofurantoin, which is virtually inactive at tissue levels Nitrofurantoin may be used for prolonged periods of time with relative safety, although the incidence of paresthesias, gastrointestinal disturbances, and peripheral neuropathy is somewhat high Unfortunately, many strains of bacteria that are found in chronic infections of the urinary tract are resistant to this drug, as well as to many antibiotics.

Ideally, for a drug to be useful in suppressing bacteriuria, it should be nontoxic, be active in urine, be active against all of the common pathogens of the urinary tract, have low mutagenicity, and not interfere with the action of antibiotics if the latter should prove to be necessary because of evidence of spread of infection. A review of available drugs had indicated that some of the older substances such as hexamethylenetetramine and mandelic acid merited reinvestigation

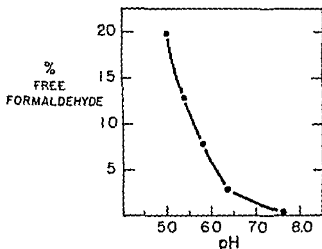


FIGURE 1 Effect of media pH upon the release of formaldehyde from hexamethylenetetramine

Hexamethylenetetramine or methenamine has the property of releasing formaldehyde, it is readily excreted in the urine, but unless the pH of the urine is carefully controlled, insufficient amounts of formaldehyde are released for antibacterial action to be manifest (Figure 1) Garrod has recently undertaken a systematic review of the use of this substance, and it may be hoped that useful therapeutic information will emerge*

Similarly, the antibacterial action of mandelic acid, as well as of other organic acids, varies with the pH of the medium, being more active at lower pH values Several authors have studied this problem and have

be depressing if this approach were not superseded within a few years

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tinued for a total of nine months. After treatment, her urine remained sterile for about one year. Bacteriuria then returned spontaneously, accompanied by fever. She was treated for another month in the same way as before. She has since gone one and one-half years without recurrence of bacteriuria and without symptoms.

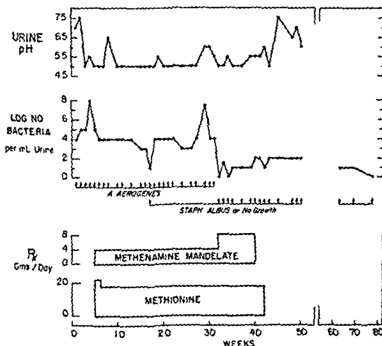


FIGURE 4 Effect of prolonged administration of dl-methionine and methenamine mandelate in a patient with chronic pyelonephritis. Note the effect of increasing the dose of methenamine mandelate at the thirtieth week of treatment

A second patient (Figure 4) had a five-year history of chronic pyelonephritis, and a pyeloplasty for bilateral hydronephrosis. Persistent bacteriuria and nocturia were of such severity that his marriage and employment status were seriously threatened. Methionine was given with methenamine mandelate. The urine pH fell, but despite a decreased level

such as hippuric acid, in urine is such that under normal dietary conditions there may be sufficient amounts of organic acids to bring about bacteriostasis if the pH of the urine is low enough. Methionine alone does not supply prolonged urinary bacteriostasis.

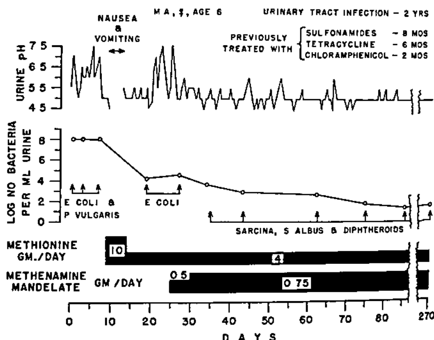


FIGURE 3 Effect of prolonged administration of dl-methionine and methenamine mandelate in a child with chronic urinary tract infection

However, with sufficient lowering of the pH of the urine, and sufficient elevation of the content of organic acid, it should be possible to make the urine unable to support the multiplication of any of the common urinary pathogens. Figure 3 shows the results of using methionine in conjunction with methenamine mandelate in a child with a two-year history of chronic infection, with failure of four previous courses of treatment.[9] She was taught to record her urinary pH values, using nitrazine paper, and to adjust the intake of methionine to the smallest dose that would keep the urine pH at about 5.0. Promptly after the beginning of treatment the bacterial count of her pathogens fell, and after about a month, the pathogens disappeared from her voided specimens. The treatment was con-

that bacteriuria has not returned after cessation of treatment No patient with chronic urinary tract infection has been accepted for treatment who has not had at least two well-documented previous antibiotic failures, therefore, the results of applying the principles of urinary acidification and urinary antisepsis in such patients indicate that we can offer a more hopeful approach to long-term management

There are substantial disadvantages to this form of treatment Methionine is not especially palatable, and often is associated with eructations and similar gastrointestinal complaints Clinical acidosis may occur, particularly in the presence of renal insufficiency The doses of drug are large, and the demands on the patient in terms of measuring pH and regulation of dosage are substantial Bacterial counts of voided urine must be performed regularly in order to check on the progress of therapy However, the approach has been useful in otherwise hopeless clinical situations

It is conceivable that improved methods for the control of urinary pH and the development of antibacterial agents with higher pK values will make the task easier With further knowledge, a clearer definition of the length of treatment may emerge At present, we treat for three to six months – and if bacteriuria recurs, treat for an additional six-month period

The data of Beeson, Rowley, and Freedman, in this symposium, indicate that persistent acidosis may influence adversely renal defensive mechanisms Although the kidney may thus become susceptible to infection, it is conceivable that antibacterial action keeps bacteria out of the susceptible tissues

In summary, it can only be said that prolonged antibacterial treatment, by whatever means, required careful and continued study of the patient, with frequent bacterial counts of the urine Suppression of bacteriuria may be achieved by various means and, when successful, may over a period of months lead to eradication of the chronic infection in many patients The reasons why the infection is not eradicated in some patients and the mechanisms by which the urinary tract spontaneously rids itself of infection in other situations require further study

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of bacteriuria the pathogens persisted. The dose of methenamine mandelate was therefore increased to a level that would, possibly, in itself cause dysuria, and the patient was so informed. The bacteria disappeared from the urine. After the high dose of methenamine mandelate had been maintained for about two months, both drugs were discontinued The urine remained free of pathogens. For the past two years, the patient's *urine has been sterile and his economic and marital status are much* improved

The experience with these forms of treatment is not great Table II shows the results of treatment in patients given methionine and methenamine mandelate.

TABLE II. Chronic Infection of Urinary Tract Treated with dl-Methionine and Methenamine Mandelate

	Colony Counts per Milliliter of Urine*						
pH of Urine	$>10^6$	10^5	10^4	10^3	10^2	10^1	0
4 5–5 0	0	1	1	5	2	0	8
5 0–5 5	2	0	2	0	1	0	1
>5 5	4	1	0	0	0	0	0
Total patients	6	2	3	5	3	0	9

* *After treatment All patients had $>10^6$ bacteria per milliliter before treatment*

mine mandelate. The patients whose urinary pH could be lowered usually showed a therapeutic response Most failures have been due to the presence of *Proteus* infections with alkaline urines, although in a few instances even alkaline urines have been acidified and control of *bacteriuria* has been achieved Similar results have been obtained using hippuric acid and methionine (Table III) Nitrofurantoin has also been used in prolonged treatment

TABLE III. Chronic Infection of Urinary Tract Treated with dl-Methionine and Hippuric Acid

	Colony Counts per milliliter of Urine*						
pH of Urine	$>10^6$	10^5	10^4	10^3	10^2	10^1	0
4 5–5 0	0	1	2	1	0	0	0
5 0–5 5	0	0	1	0	0	0	0
>5 5	2	0	0	0	0	0	0
Total patients	2	1	3	1	0	0	0

* *After treatment All patients had $>10^6$ bacteria per milliliter before treatment*

At no time that the bacteriuria has been maintained below 10^4 colonies per milliliter has a patient developed symptomatic infection of the urinary tract. In about half of the cases, prolonged follow-up has shown

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*The Present Status of the Chemotherapy of Pyelonephritis**

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Within a brief period of less than fifteen years there have been discovered, made available, and put into wide use a succession of synthetic chemicals and antibiotic agents that offered every reason to expect that infectious diseases caused by bacteria, rickettsias, and large viruses would no longer provide any difficult therapeutic problems, and indeed that many of the most serious among them might even be prevented. Even before that, the introduction of methenamine and the principle of acidification, as exemplified by the ketogenic diet and mandelic acid, held out considerable hopes for the simple and successful management of infections of the urinary tract, and these hopes were considerably strengthened by the availability and use of the newer chemotherapeutic agents that seemed to be more active, and cover a wider range, if not all, of these infections.

The intervening years have proved that these expectations have not been entirely fulfilled, and this in spite of the feverish activities of a large number of our leading pharmaceutical manufacturers and their research staffs which have succeeded in discovering tremendous numbers of new agents and in introducing into the market many of them, of which some are considerably better than their predecessors, and others not as good. Indeed the use of all of these agents, or perhaps their excessive use and abuse, seems to have created new problems or problems of greater complexity. In all fairness and to avoid gross misinterpretation, it must be stated at the outset that antimicrobial agents are still responsible for the saving of countless lives and the curtailing of a tremendous amount of serious and debilitating illness.

At the 1959 meeting of the Association of American Physicians I presented data, collected at the Boston City Hospital with the help of Wilfred F. Jones, Jr., and Mildred W. Barnes, which show the changes that have

* From the Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical Services, Boston City Hospital, and the Department of Medicine, Harvard Medical School. Aided by a grant (E-23) from the National Institutes of Health, United States Public Health Service.

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the staphylococcus, were already available and being used, and finally 1953, 1955 and 1957, three years in which all the modern antibacterial armamentarium, except for a few special ones such as vancomycin, ristocetin, and kanamycin, was being used under what might be considered reasonable supervision, presumably with some precision, and according to what may be considered reasonable, if not the best, precepts

It is seen that patients with pneumococcal bacteremia (open bars on the left of each group) were encountered in considerable numbers throughout the entire period with a slight reduction in their number during the last two years that were surveyed There were about half as many patients with hemolytic streptococcal as with pneumococcal bacteremia in 1935, but the number of hemolytic streptococcal bacteremias decreased markedly and only small numbers have been encountered since then, although they began to reappear in 1957 and seem to have been increasing somewhat since then. The incidence of *Streptococcus viridans* bacteremia, represented by the middle, horizontally crossed bars, showed no particular trend On the other hand, patients with bacteremia due to enterococcus were not encountered in 1935 and were rarely seen before the antibiotic era, but there have been 25 to 36 such patients each year since then Patients with *Staphylococcus aureus* bacteremia were encountered either less frequently or about as often as those with pneumococcal bacteremia until 1951, since that time the number of patients in whom *S aureus* was grown from the blood increased markedly and steadily, whereas the number with pneumococcal bacteremia declined appreciably In 1957, there were nearly four times as many patients with *S aureus* bacteremia as in 1935, and more than twice as many as in 1947

The numbers of deaths in these cases of bacteremia due to gram-positive cocci reflect the activity of the antibacterial agents that became available (Figure 2) Deaths in patients with pneumococcal and hemolytic streptococcal bacteremia declined progressively with the successive introduction of the sulfonamides and penicillin and the later antibiotics, with only a relatively small number of deaths still occurring among patients with pneumococcal bacteremia and almost none among those with hemolytic streptococcal bacteremia There was some reduction in the number of deaths in patients with *Str viridans* bacteremia but they still occur in moderate numbers Fatal cases of enterococcal bacteremia, however, were not encountered in 1935, but there have been from 5 to 15 such deaths each year since 1947 Deaths in patients with *S aureus* bacteremia declined in number after the introduction of penicillin, but since 1947 the number of such deaths increased steadily, in fact, they increased about fivefold in the ensuing 10-year period covered in this study, reflecting not only the increasing number of patients but the decreasing effectiveness of therapy.

occurred in the incidence and mortality of serious infections associated with common bacterial species at that hospital since the introduction of the modern, highly active antimicrobial agents. The paper[6] has been published recently in the *Journal of the American Medical Association*, but I ask your indulgence as I present some charts from that paper, they

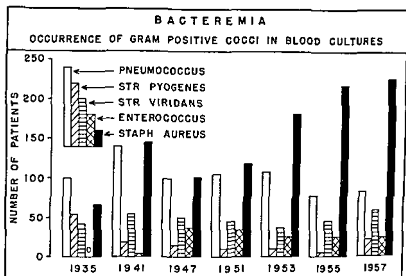


FIGURE 1 * Data from Boston City Hospital reported by Finland *et al*[6]

deal only with patients in whom infections were accompanied by blood stream invasion, but they have a direct bearing on the problems under discussion

The first of these (Figure 1) shows graphically the number of patients with infections associated with blood stream invasion by various gram-positive coccal organisms in seven different years These years were specifically chosen because of their relation to the successive introduction of our most active antibacterial agents 1935 represents a year just before the first introduction here of sulfanilamide and its derivatives, 1941 was a year in which the most active of the sulfanilamide derivatives, namely sulfadiazine, was very extensively used but before the introduction of the antibiotics, 1947 is a year in which both penicillin and streptomycin were already widely and intensively employed but before the broad-spectrum antibiotics became available, 1951 represents a year in which all the major antibiotics, except those directed specifically against

* Figures 1-4 are reproduced from Finland *et al*[6] with permission of the editor and publisher of the *Journal of the American Medical Association*

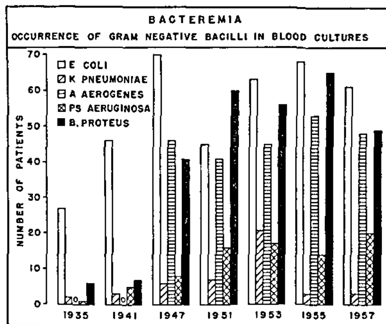


FIGURE 3 Data from Boston City Hospital reported by Finland *et al*[6]

the same proportion (Figure 4), since the case fatality rate in each instance was from 40 to 60 per cent. In each of the last three years that were surveyed there have been about 100 deaths in patients with bacteremia due to these gram-negative bacilli

It is fair to say, although this has not been completely documented, that a significant proportion, if not the great majority, of these cases and deaths were associated with corresponding infections of the urinary tract Moreover, it is just these organisms, notably *Aerobacter*, *Proteus*, and *Pseudomonas*, which are found most frequently in chronic and complicated cases of urinary tract infection in which the available antibacterial agents have been used extensively and intensively, but with the least success[1, 2, 7, 13, 15, 17]

Detailed information concerning the individual antimicrobial agents or therapeutic regimens, the frequency with which various bacterial species are implicated under specific conditions and in the different types of infections of the urinary tract, and the results that have been achieved in the application of the available therapeutic agents to the treatment of such infections were all reviewed in 1955 by Kass,[10] and the available

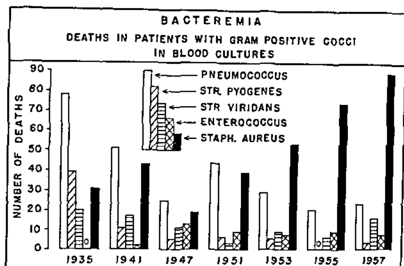


FIGURE 2 Data from Boston City Hospital reported by Finland *et al*[6]

Needless to say, all these changes correlate well with the effectiveness of the antibacterial agents that have become available and which manifest their greatest activity against the pneumococcus and hemolytic streptococcus, less against strains of *Str. viridans*, with *S. aureus* and to some extent also enterococci manifesting increasing resistance to most of the successive agents as they came into wide use.

More relevant to the subject of this paper and of this symposium, however, are the data on infections that are accompanied by invasion of the blood stream with the common gram-negative bacillary organisms, notably *Escherichia coli*, *Aerobacter aerogenes*, *Proteus* and *Pseudomonas*. In Figure 3 it is seen that in 1935, before the sulfonamides became available, *E. coli* was responsible for a moderate number of cases of bacteremia and a small number of patients with *Proteus* bacteremia were encountered in that year, there were occasional patients with blood cultures positive for *Klebsiella* (Friedlander's bacillus) or *Pseudomonas*, and none with blood stream invasion by *Aerobacter* were encountered. Since that time, there have been only minor fluctuations in the occurrence of *Klebsiella* bacteremias, but blood stream invasion by each of the other gram-negative bacilli shown here has increased markedly, the number of patients with *Pseudomonas* bacteremia increased to about 20 a year, and during each of the years of this study, beginning in 1947, there have been from 40 to more than 60 patients with blood stream invasion due to each of the other three organisms, *E. coli*, *A. aerogenes*, and *Proteus*. The number of deaths in these patients with gram-negative bacillemia increased essentially in

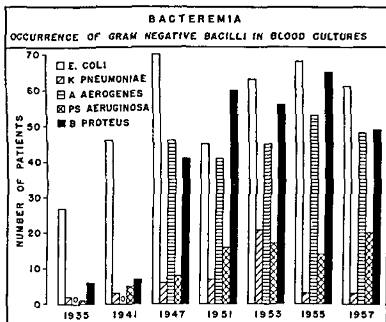


FIGURE 3 Data from Boston City Hospital reported by Finland *et al* [4]

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information has been brought up to date by Hewitt in a review on pyelonephritis prepared in collaboration with Kleeman and Guze.[12] It is not my intention to cover any of these details, nor would any good purpose be served thereby.

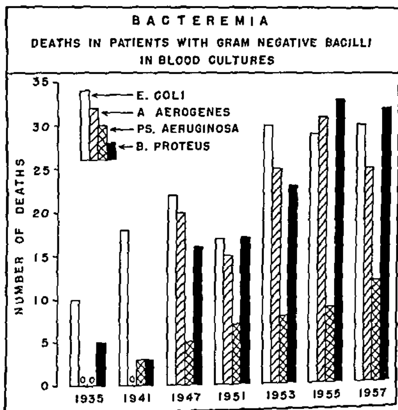


FIGURE 4 Data from Boston City Hospital reported by Finland *et al* [6]

Perhaps the most striking feature brought out in these reviews, and even more in reading the successive detailed reports from the same clinics or from different clinics on the use of any given agent, or on all the agents as they became available, is the similarity and the more or less repetitive character of the results. The initial or early reports of each new agent or regimen are generally marked by favorable results and by enthusiasm engendered by the differences in mode of action, spectrum of activity, untoward effects, or ease of administration, which permit the investigator almost invariably to record initial successes in patients who previously failed to respond favorably or adequately to single or multiple agents

given to the same patients for the same episode or for similar previo episodes. As time goes on and further experience with the same agen is acquired, they somehow lose their charm and the results, on the who fall into the same pattern, varying only with respect to some specifi activity of the particular agent or with the types of infections or condi tions against which they are pitted.

The strictly antibacterial agents have generally, but not invariably, been effective in reducing the number of organisms in the urine or totally eliminating infections with organisms that can be shown to be susceptible *in vitro* to the concentrations of the same active agent achieved in the tissues or in the urine. Correlations of bacteriologic successes with *in vitro* susceptibility of the organisms obtained in cultures have ranged, in different reports, up to 90 per cent, or even higher, when single organisms are involved in uncomplicated acute episodes of infection. The incidence of successes is generally reduced by one-third to one-half by the presence of complicating obstructive or destructive lesions within the urinary tract, and still further by previous therapy with the same or other agents, since this generally implies chronicity of the infection and also instrumentations and manipulations of the urinary tract, multiple catheterizations or the use of an inlying catheter — all of which permit the introduction of new bacterial agents. This has been true of the use of each of the successive chemical and antibiotic agents that have come into use In the types of patients in whom repeated failures have followed intermittent or temporary successes, the failures have almost invariably been associated with the presence, emergence, or introduction of bacteria that are resistant to the successive agents that have been used.[2, 5, 8, 9, 16]

By any broad definition of chemotherapeutic agents which would encompass all those used in the treatment of urinary tract infections, these agents act only on the infecting bacteria, whether in the urine or the tissues, to inhibit their multiplication and, for some, to kill the organisms and thus eliminate them as a cause of the infection From this point of view, practically all of the agents, whether used singly or in combinations, may be said to be accomplishing essentially what can be expected of them from their known activities. This, however, is being accomplished within rather important limitations imposed, in part, by the bacteriologic, pharmacologic, and physical properties of the agents that are available, and even more by the host and the environmental conditions provided by it and by the infected area as the substrate for the infecting bacteria and for the action of the agents which are offered to attack them Thus, the action of acidifying agents appears to be successful in a large proportion of cases in which it has been possible to achieve the necessary degree of acidity and to maintain it long enough to clear an acute uncomplicated infection with an organism that is sufficiently inhibited by the degree of

acidity that is maintained. However, these agents have been limited on the one hand by the ability of the patient to attain and maintain long enough, at the infected site, the acidity that is required, and on the other hand by the occurrence of organisms that can survive and even multiply in an acid medium or which defeat that purpose by producing ammonia and maintaining an alkaline reaction in the urine.

Among the more strictly antibacterial agents, some are rendered inactive, or their activity is reduced, because the medium is not optimum, or inadequate concentrations are achieved at the site of the organisms, or the concentrations of organisms are too great or are inaccessible to the antibiotic, or because highly resistant mutants emerge (the latter most strikingly illustrated in the case of streptomycin). Most important, however, seems to be the fact that, as increasing numbers of patients are exposed to treatment with antimicrobial agents, their susceptible organisms are eliminated or inhibited from multiplying, leaving the more resistant ones to flourish. For this reason, in the hospital environments and, perhaps, in the offices of busy practitioners in which these patients are treated and manipulated, these resistant organisms have the greatest opportunity to spread, directly or indirectly, to other patients Thus we find the predominance of certain strains or species that become characteristics of certain hospitals or areas, such as *Klebsiella* type 8 in the Copenhagen area,[14] *Achromobacter* in Seattle,[11] and *Proteus*, *Aerobacter*, and *Pseudomonas* in most other places, and these appear and persist mainly in the chronic, complicated cases.[1, 2, 13, 15, 17]

The question may be asked whether the use of antibacterial agents does not actually interfere with the natural protective responses of the host to the invading bacteria. This may be true, or is at least conceivable insofar as some of them injure tissue or disturb local or general metabolic balance, or produce specific toxic effects, but in general the agents that are used have been preselected because they do not act in this way. Interference with immune mechanisms in the host is another possibility that must be considered. These are poorly understood as regards their role in the types of infection with which we are concerned, but they probably play only a very minor and unimportant role, this aspect deserves further study and is in fact being intensively investigated in some quarters, as illustrated already in this symposium On the other hand, the elimination of susceptible strains which disturbs the normal ecological balance among various bacteria in the "normal" flora of the body or of its environment may have permitted others that are normally few in number and of low pathogenicity to multiply uninhibited by the antibacterial agents until they achieve virulence, either through serial passages in successive hosts or merely by force of numbers Just where they come from and how they produce infections in the urinary tract is not

entirely clear but is now receiving some attention and deserves further intensive study.

Where do the chemotherapeutic drugs achieve their decisive effects in eradicating urinary tract infections? Is it in the infected tissues of the kidney or other structures of the genitourinary tract which serve as the reservoir of the inoculum for continuous infection of the urine, the latter being only a reflection of the continued infection of the tissues? Or is it in the infected urine which serves as a source of infected and continued reinfection of the tissues, because the urine is such a favorable medium for heavy growth of the bacteria? This would appear to be of more than theoretical importance for, as was illustrated by Kunin in his presentation, some antibacterial agents produce relatively high levels in the blood, and presumably in the tissues, and only relatively small amounts of the active drug are excreted into the urine, whereas others produce only low and perhaps ineffective concentrations in the plasma which might not be active in the tissue sites of bacterial invasion but are excreted primarily or predominantly into the urine, where high concentrations of active drug are achieved, making possible a greater range of effectiveness against bacterial pathogens in the urine

Some recent evidence obtained from studies on the effects of sulfonamide drugs would indicate that the tissue levels are the more significant, this is illustrated by the activity of sulfamethylpyridazine, which produces and sustains much higher concentrations of free sulfonamide in the plasma and significantly lower concentrations in the urine than can be achieved with more than four times equivalent doses of sulfisoxazole[18] Another group of observations,[3,4] on the other hand, suggest that neither of these sites is crucial in the eventual clearing of chronic urinary tract infections, since favorable results may be achieved by prolonged administration of the relatively unabsorbed or poorly absorbed sulfonamides, sulfasuxidine and sulfathalidine, negligible concentration of active drugs results in the blood from their use in moderately large oral doses and the concentrations achieved in the urine would prove grossly inadequate if they resulted from the very small doses of sulfathiazole that could provide such urinary levels. However, since the results achieved with all of these agents still leave much to be desired, these findings cannot be taken as providing acceptable answers to the question posed These can come only from the types of detailed study of pathogenesis and therapy that have been presented in these sessions and by further study, both clinical and experimental.

Whatever the final answer to these questions may be, if indeed any single definitive answer will be found, certain practical implications follow from the type of results that have been reported here and elsewhere by Kass and his associates These results stem originally from the concept of

the significance of "inapparent infection" of the urinary tract as revealed by demonstration of the so-called "significant bacteriuria" as determined, in turn, by routine quantitative bacteriologic examination of the urine From the preliminary results achieved thus far in the prevention of clinical manifestations of active infection of the urinary tract by relatively simple treatment of the inapparent ones that have been uncovered in this way, it would appear that the search for and discovery of "significant bacteriuria" and the achievement and maintenance of a sterile urine may, by itself, prove to be the optimum way in which to prevent active acute infections or recurrences of episodes of such infections.

Those of you who have had the patience and persistence to sit through the sessions of these past three long and full days really deserve a happier and more encouraging concluding presentation than I have had to offer. However, if the showing to date, insofar as it concerns the results of the application of antibacterial therapy to urinary tract infections in man, has not been very brilliant and the current status is far from desirable, we may at least take courage from the fact that it has not led to complacency. The very fact that these failures, or limited successes, have stimulated so many brilliant investigators to delve more deeply into this subject justifies the hope that new and more fruitful methods of management and prevention of urinary tract infection may evolve in the not too distant future

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GENERAL DISCUSSION

DR. DOOLAN: When we were studying amino acid reabsorption we had occasion to use high doses of methionine, and I had the feeling that this could be quite toxic. I wonder if Dr. Kass would comment.

DR. KASS: I don't quite know what you mean by toxicity in this case, Dr. Doolan, but people vary considerably in their response to methionine. Most people don't like the way it tastes and smells, but they tolerate it reasonably well at levels of 10 or 12 Gm. a day; a few have developed gastrointestinal disturbances such as nausea and vomiting. One or two have had mild diarrhea. One patient said that his teeth got softer, he thought, and one asked me whether the fact that his hair was falling out had anything to do with methionine. In the literature there are such depressing things as failure of testes of rats to develop properly when huge doses of methionine were *fed to very young rats. I have no idea what this* means.

This is far from ideal treatment, but is the best that we can offer at the moment.

DR. DAVIS: Dr. Kass and his disciples believe earnestly that instrumentation of the urinary tract is dangerous and should not be done if there is any way to avoid it. This belief is based on the concept that it may initiate symptomless bacteriuria which may lead to nonobstructive pyelonephritis. I think that obstruction is often symptomless, slight, occult and hard to find. *I think the existence of nonobstructive pyelonephritis has not been* proved. Since nonobstructive pyelonephritis can be proved only by exclusion, I think all interested in this disease should utilize all of the rapidly increasing and improving means of determining whether or not obstruction is present. A stricture at the mouth of the collecting tubule is of great interest to the renal pathologist, but we now know that a ureteropelvic obstruction may have the same effect.

My position is, if you like, as extreme as that of Dr. Kass, and I assure you just as conscientious. The great and fundamental difference is that obstructive pyelonephritis is a curable disease. Nonobstructive pyelonephritis is a disease of which, at best, and I quote Dr. Kass, "80 per cent of the cases are incurable."

DR. GRIEBLE: What is the rationale for the selection of a treatment period of three to six months? With Dr. Jackson, we have shown that using sulfamethoxypyridazine, nitrofurantoin, or certain *antibiotics there* were no more cures, as judged by bacterial counts in the urine, whether treatment was given for five to ten days or for periods of time up to six or

ten months. If one considers the return of bacteriuria after treatment as failure and elimination of significant bacteriuria as successful treatment, there appears to be little merit in the long-continued use of a suppressive agent as opposed to short-term treatment with a specific effective antibiotic. Neither regimen is highly successful, but we prefer multiple short courses with a specifically selected drug

DR. YOW: I should like to comment on Dr Fisher's paper We have recently carried out some experiments in which we have produced hematomas artificially in rabbits and infected these hematomas with staphylococci. Our experience was somewhat similar to that of Dr Fisher in that if we treated the rabbits systemically we could sterilize these abscesses if treatment was begun during the initial four or five days Beyond that point no amount of systemic antibiotic had any influence on the cultures of the infected hematomas On the other hand, if the antibiotic was injected directly into the hematomas, we could sterilize the hematoma. We feel this suggests that something in the healing process isolates the infection from the blood stream, and consequently from the effects of antibiotics that get there by way of the blood stream.

DR. BROD One thing that comes out clearly from this symposium is that the pessimism as regards chronic pyelonephritis is a matter of the past, and that we can view the future with much more optimism I can't remember a single patient with pyelonephritis dying in the past two years. I think this is due to antibiotic and chemotherapeutic treatment As we have eradicated infection in many of our patients the glomerular filtration rate has risen and become stationary In one patient we controlled the infection for about three years We then lost contact with the patient. Subsequently, the infection became very severe, and the glomerular filtration rate went to zero and the patient died. This shows that we must use therapy very intensively I do not agree that only five days of therapy would be helpful I think we must administer antibiotics for months and then make a change – probably to some chemotherapeutics and to some of the older agents

I should like you to answer, Dr Finland, and also Dr. Kass, whether the acidification of which you spoke can be produced in patients with a severe pyelonephritis, because there we know it is very difficult to change the reaction of urine I think these good results which I have just mentioned make one thing imperative – that we do not let these patients go on until they have a glomerular filtration rate of 20 or 15. We can keep them alive, but certainly it is a restricted life, and the question of early diagnosis is imperative

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usually eliminate the organism that is sensitive to a given drug by the use of that drug. However, we generally end up with an organism that is resistant. Whether new organisms are constantly being reintroduced into the infected urinary tract, whether this is merely a reflection of the fact that most people with chronic infection of the urinary tract don't have pure cultures and therefore a suppressed organism emerges, or *whether perhaps other factors about which we know nothing are operative*, of course we can't answer However, we can say that in the situation Dr Petersdorf posed, most of the people who do get bacteria back when they have been given an effective drug have an organism of different antibiotic sensitivity from the one they started with

The factor of increased susceptibility of the infected urinary tract to reinfection is one that we have not known much about In some of our studies and some of those that Dr Fisher has done (and I am sure others who have worked with the experimental infection have observed the same thing), there is an interesting phenomenon that seems relevant. If we put *Escherichia coli* into the bladder of rats without any glass bead, the *E. coli* tend to disappear quickly If a glass bead is put in place to assure a little residual urine in the bladder, the *E. coli* tend to persist but very little pyelonephritis is produced by this organism However, if these animals are left alone under normal laboratory conditions, a certain number of them pick up *Proteus* infections spontaneously and develop pyelonephritis. Every instance so far of clear-cut severe pyelonephritis in the animals that had *E. coli* and glass beads in the bladder turned out to be due to superinfection with *Proteus*, and none of this happened within the first month after the *E. coli* and glass beads had been introduced.

Dr. Fisher has similar data in the enterococcic system showing superinfection with a gram-negative rod. So there may be a question here of whether the previous infection in some way paves the way for the ready establishment of new bacterial species, perhaps by interfering with local defensive mechanism of the type Dr. Beeson and Dr. Rowley were discussing At least these are areas for investigation.

With respect to Dr. Grieble's point: my impression of the sulfonamide studies was that this was the type of situation I just described—that the organisms when sensitive were removed and in a small number of patients this led to bacteriologic cure, but the majority did not have a cure for the reasons I have cited.

The added factor in the use of organic acids is that these are much less selective than are antibiotics. So far we have not encountered any gram-negative rods that differ from other gram-negative rods by a factor of more than twofold in terms of susceptibility to organic acids Thus, all the bacteria in a mixed flora are suppressed rather than one strain Almost all of our patients are people who have sulfonamide-resistant,

How can we come to an early diagnosis? Certainly we can't do it by screening bacteria in every person who comes to us. I think there are two things which should draw our attention to possible kidney disease: first, the presence of proteinuria – that is, proteinuria that we find in a specimen voided in the morning; and secondly, otherwise unexplained hypertension in a young subject. If these two things are found in a patient on casual examination, the patient should be referred to a department where he can be properly examined; and then if the functional methods are used, I think the diagnosis can be arrived at in about 90 per cent of the patients.

My last remark would answer one question that Dr. Kleeman asked yesterday. I don't think we can do only one of these diagnostic procedures – such as just doing a comparison of the glomerular filtration rate and concentrating power. We have to use all the functional tests that we have in our hands, together with radiology, and then out of these complex approaches we can arrive at the correct diagnosis.

DR. FINLAND Dr. Brod, I think you defeated your own purpose by carrying on after you asked your question, because your procedure is so elaborate that it is impossible to screen people until after they have developed some of the adverse consequences of pyelonephritis. Every doctor who examines a patient completely does a urinalysis, and it is just a question of learning how to adapt the present method of urinalysis to include screening for bacteriuria, which is one of the earliest signs and perhaps the first sign that might be obtained.

DR. PETERSDORF: Dr. Kass, if one does tube dilution sensitivity tests on a number of urinary pathogens, one finds they not only are inhibited but also are sometimes killed by concentrations which are usually not present in the usual discs. Thus, a number of organisms which are considered resistant might indeed be sensitive, and the antibiotic in the urine could indeed be shown – and has been, in a number of instances – to be present in sufficiently high concentrations to exert its bacteriostatic or bactericidal action.

If it is true that the urine is the focus for reinfection of the kidney, I should like to ask Dr. Kass why we see so many therapeutic failures in instances where the antibiotic is present in rather high and presumably adequate concentrations in the urine.

DR. KASS· Perhaps I can take these questions in reverse order, since I think this will lend itself to an easier discussion

I wish I knew the answer to Dr. Petersdorf's question On the basis of the data, I think we can say that, as Dr. Finland mentioned, we can

usually eliminate the organism that is sensitive to a given drug by the use of that drug. However, we generally end up with an organism that is resistant. Whether new organisms are constantly being reintroduced into the infected urinary tract, whether this is merely a reflection of the fact that most people with chronic infection of the urinary tract don't have pure cultures and therefore a suppressed organism emerges, or whether perhaps other factors about which we know nothing are operative, of course we can't answer. However, we can say that in the situation Dr Petersdorf posed, most of the people who do get bacteria back when they have been given an effective drug have an organism of different antibiotic sensitivity from the one they started with

The factor of increased susceptibility of the infected urinary tract to reinfection is one that we have not known much about. In some of our studies and some of those that Dr Fisher has done (and I am sure others who have worked with the experimental infection have observed the same thing), there is an interesting phenomenon that seems relevant. If we put *Escherichia coli* into the bladder of rats without any glass bead, the *E. coli* tend to disappear quickly. If a glass bead is put in place to assure a little residual urine in the bladder, the *E. coli* tend to persist but very little pyelonephritis is produced by this organism However, if these animals are left alone under normal laboratory conditions, a certain number of them pick up *Proteus* infections spontaneously and develop pyelonephritis Every instance so far of clear-cut severe pyelonephritis in the animals that had *E. coli* and glass beads in the bladder turned out to be due to superinfection with *Proteus*, and none of this happened within the first month after the *E coli* and glass beads had been introduced

Dr. Fisher has similar data in the enterococcic system showing superinfection with a gram-negative rod So there may be a question here of whether the previous infection in some way paves the way for the ready establishment of new bacterial species, perhaps by interfering with local defensive mechanism of the type Dr Beeson and Dr. Rowley were discussing At least these are areas for investigation

With respect to Dr Grieble's point my impression of the sulfonamide udies was that this was the type of situation I just described — that the ganisms when sensitive were removed and in a small number of pati-- s led to bacteriologic cure, but the majority did not have a cure reasons I have cited

The added factor in the use of organic acids is that these are mu selective than are antibiotics. So far we have not encountered a n-negative rods that differ from other gram-negative rods by a fact ore than twofold in terms of susceptibility to organic acids. Thus, a bacteria in a mixed flora are suppressed rather than one strain st all of our patients are people who have sulfonamide-resistant

How can we come to an early diagnosis? Certainly we can't do it by screening bacteria in every person who comes to us. I think there are two things which should draw our attention to possible kidney disease: first, the presence of proteinuria – that is, proteinuria that we find in a specimen voided in the morning; and secondly, otherwise unexplained hypertension in a young subject. If these two things are found in a patient on casual examination, the patient should be referred to a department where he can be properly examined; and then if the functional methods are used, I think the diagnosis can be arrived at in about 90 per cent of the patients.

My last remark would answer one question that Dr. Kleeman asked yesterday. I don't think we can do only one of these diagnostic procedures – such as just doing a comparison of the glomerular filtration rate and concentrating power. We have to use all the functional tests that we have in our hands, together with radiology, and then out of these complex approaches we can arrive at the correct diagnosis.

DR. FINLAND: Dr. Brod, I think you defeated your own purpose by carrying on after you asked your question, because your procedure is so elaborate that it is impossible to screen people until after they have developed some of the adverse consequences of pyelonephritis. Every doctor who examines a patient completely does a urinalysis, and it is just a question of learning how to adapt the present method of urinalysis *to include screening for bacteriuria, which is one of the earliest signs* and perhaps the first sign that might be obtained.

DR. PETERSDORF: Dr. Kass, if one does tube dilution sensitivity tests on a number of urinary pathogens, one finds they not only are inhibited but also are sometimes killed by concentrations which are usually not present in the usual discs. Thus, a number of organisms which are considered resistant might indeed be sensitive, and the antibiotic in the urine could indeed be shown – and has been, in a number of instances – to be present in sufficiently high concentrations to exert its bacteriostatic or bactericidal action.

If it is true that the urine is the focus for reinfection of the kidney, I should like to ask Dr. Kass why we see so many therapeutic failures in instances where the antibiotic is present in rather high and presumably adequate concentrations in the urine.

DR. KASS: Perhaps I can take these questions in reverse order, since I think this will lend itself to an easier discussion.

I wish I knew the answer to Dr. Petersdorf's question. On the basis of the data, I think we can say that, as Dr. Finland mentioned, we can

certain antibiotic regimens were used, and perhaps, as Dr Finland mentioned, we may be dealing with an impairment of host immunity by the antibiotic.

Finally, I think we are dealing in our system with the problem of microbial persistence, that is, a parasite has attained some relationship with the host which apparently is beyond the control of the immunologic and therapeutic regimens that we have been using I think this infection we have described may serve as a useful model for studying this kind of persistence.

DR MAXWELL Dr Guze, Dr. Kleeman, and I have had 1½ years' experience in the use of methionine, Mandelamine, and mandelic acid in various types of urinary tract infections Although the data are not complete and are not completely analyzed, our experiences can be summarized as follows: (1) The use of methionine and Mandelamine offers no advantages over methionine and mandelic acid, which seems to eliminate the occasional dysuria which is evidently caused by the methenamine radical in the Mandelamine (2) The combination of methionine and mandelic acid in a single pill (Urometh-M, D'Franssia Laboratories, Los Angeles, Calif), which is flavored with mint, has helped to overcome the objection which some patients have to the large number of pills which they are forced to ingest on this regimen With this combination in a dosage of 6 to 12 Gm of methionine daily we have encountered gastrointestinal symptoms in less than 10 per cent of our patients (3) Methionine and mandelic acid have not been effective in the prophylactic therapy of patients undergoing transurethral prostatic resections These drugs were given to alternate patients, starting several days before the operation and continuing for four weeks The alternate control patients were given no prophylactic therapy, and various antibiotics were administered when indicated The incidence of bacteriuria and symptomatic pyelonephritis was essentially the same in both groups (4) We can confirm Dr. Kass' statement that the combination of methionine and mandelic acid sometimes proves highly effective in reducing the bacteriuria in patients with long-standing resistant chronic pyelonephritis and in completely eliminating the infection in others At the present time we have found no way to predict from the type of organism, the urinary sediment, or any other objective criterion, the patients in whom this form of treatment will be effective. (5) We have found consistently that methionine in the usual therapeutic dose causes a severe symptomatic metabolic acidosis in patients with azotemia, and for this reason we did not attempt its use in patients with a serum creatinine of over 3.0 mg per cent

DR HAMBURGER How does the inhibition of cell wall synthesis relate

tetracycline-resistant and usually chloramphenicol-resistant bacteria, because we will accept in our clinic for treatment only patients who have had two well-documented previous therapeutic failures Anybody can cure the easy ones; the problem is to deal with the difficult ones.

Why did we pick three or six months? In some of the treated patients the pathogens tended to persist at low levels of bacterial count for a month or two. In other instances three or six months were nice round numbers, and I can only defend it on this basis. If someone wants to show data to demonstrate the validity of a shorter time period, nothing would delight me more, and nothing would delight most of our patients more At the moment we are groping toward an approach to the problem, and as much as I share Dr. Grieble's feeling that I should have a clear rationale, our choice of the time period of treatment has been arbitrary.

With respect to Dr. Brod's question, we can't use acidifiers in patients with severe renal insufficiency except under very carefully managed circumstances. They will tend to become more acidotic. However, when there is renal insufficiency, as Drs. Schwartz and Relman showed recently, the pH of the urine tends to be substantially lower than otherwise This is in part due to reduction in the buffer content of the urine, and partly due to impairment of the mechanism of ammonia response to acidification Perhaps there are other reasons, but in any event the urine pH tends to be substantially lower in people with chronic renal disease than in those with normal kidneys.

In such patients one can frequently get away with using hippuric acid alone in graded doses. However, one must follow the patient carefully, with frequent blood CO_2 or blood pH determinations. There is no question that people will be driven into severe acidosis almost overnight by use of acidifers, and certainly one should not use them without careful bacteriologic and chemical control.

Dr. Fisher: Unfortunately time did not permit me to make a few points in my paper, and I should like very briefly to make them now.

First, although I am guilty of using it, I believe "synergism" is a badly overworked word and it becomes almost a Madison Avenue cliché. We have shown strikingly, I think, that the so-called synergism observed *in vitro* operates only under special conditions *in vivo* I think it is a very limited concept. Others might try to make us believe it is broadly applied. I don't think it is.

Second, I should have added that we have never observed development of resistance as a cause of therapeutic failure in our system.

Third, as Dr. Kass mentioned, we observed a spontaneous superinfection with *Aerobacter* once the enterococcus was either eradicated or suppressed. This spontaneous gram-negative infection came in only when

on the susceptibility of the kidney to hematogenous spread of bacteria. Furthermore, the effect of obstruction is most notable shortly after the obstruction has occurred, as the obstruction persists, the susceptibility of the kidney to infection diminishes.

Most patients with initial episodes of acute pyelonephritis have no evidence of obstruction The demonstration of abnormal hydrodynamics [illegible]

that small degrees of partial obstruction may be overlooked with present methods of detection – at some point the argument is reduced to scholastic fineness of detail. In any event, the evidence that incomplete obstruction alters greatly the likelihood of bacterial multiplication in the kidney is remarkably inadequate.

Regardless of the degree to which partial obstruction influences susceptibility to pyelonephritis, the mechanisms by which complete obstruction alters resistance to bacterial multiplication are not understood, and conversely, the normal antibacterial mechanisms of the urinary tract are just beginning to be understood, and the experiments of Gorrill, of Guze, of Braude and his associates, and most notably the experiments of Beeson and Rowley, are beginning to indicate the complexity of the antibacterial mechanisms in the kidney The latter workers, by showing an anticomplementary effect of ammonia in the kidney, offer an insight into the peculiarities of the kidney in permitting bacterial multiplication, and also offer one of the few documented instances in which inhibition of a bactericidal mechanism has been effected by alteration of complement activity

Even when some solution has been found to the problem of determining antibacterial mechanisms *in the urinary tract, there will still remain the* problem of how bacteria reach the affected part From the present data, only general views of the pathogenesis of pyelonephritis may therefore be attempted. Such an attempt will now be made, although it is admittedly conjectural and controversial.

It is understandable that the author will utilize bacteriuria as his focal point There can be no doubt from the evidence available that bacteriuria plays a key role in the pathogenesis of pyelonephritis How bacteria enter the urinary tract is entirely unclear. The obvious pathways of entry are upward through the urethra or periurethral lymphatics, and downward after hematogenous involvement of the kidneys. The latter route seems to be unlikely save in instances of *frank bacteremia*, and the persistence of bacteriuria in the absence of evidence of renal involvement makes direct ascension via the urethra the acceptable alternative. The urethral route, of course, also explains best the increased incidence of bacteriuria

the incidence of certain infections *even more* than have immunization and antibacterial *therapy. From this thesis it follows* that in certain diseases, regardless of the value of specific *prophylactic* and therapeutic measures, long-term morbidity *figures best reflect* the socioeconomic patterns of the community.

However, other infections are less commonly associated with major community outbreaks *and seem not to follow* this pattern of association with socioeconomic status.

Pyelonephritis, probably staphylococcic infections, *and certain other infections seem to* be *a* group whose incidence is apparently little influenced by socioeconomic factors. Such infections are *generally caused by* agents that are indigenous to the host, and their *emergence as pathogens* carries with it not only the selection of occasional strains from the "normal *host flora*" of somewhat greater pathogenicity than the remaining organisms but also the implication of diminished host resistance *to indigenous* organisms. The circumstances most often associated with *increased host* susceptibility, such as debility, immaturity, surgery, and so forth, are those *in which* the prophylactic or therapeutic use of antibacterial agents is common, so that the emergence as pathogens of organisms comprising the so-called "normal flora" of the body often carries with it the additional feature that the emergent bacteria are resistant to commonly used antibacterial drugs.*

Such generalizations are admittedly oversimplifications and may therefore overlook vital relationships that are found in the omitted details They do, however, suggest that infectious diseases of the future will follow different epidemiologic and clinical patterns from those of the past, and indicate that pyelonephritis may be a prototype illness for the altered patterns of the future.

Oversimplification is a difficult hazard with which to cope. The danger of excluding, by the nature of the abbreviation involved, many details that might provide a basis for a new approach to a problem is well recognized and must be balanced against the obvious value of a concise statement of a principle. Perhaps oversimplification in pyelonephritis has reached the point at which the net loss has begun to exceed the net value of the abbreviation that "obstruction predisposes to infection" This doctrine has been accepted so widely that it rarely becomes a subject for analysis. The detailed study of the doctrine discloses some unsatisfactory features.

There can be no doubt that obstruction in the urinary tract favors the multiplication of bacteria However, partial obstruction produced experimentally, as Guze and Beeson have shown, has relatively little influence

* [illegible] oint of view, is represented by polio-
[illegible] a problem to the *community* as
[illegible] nic status) improves.

manifest disease in the urinary tract. The implications of the studies of Edwards and of those cited by Rantz in his discussion are unmistakable. Detailed biologic study of strains of organisms obtained from patients with pyelonephritis is urgently needed, and at present we have no adequate markers for discerning biologic differences that relate to pathogenicity outside of the infected host.

The upward spread of bacteria to the kidney may be by lumen or lymphatic. The careful studies from Murphy's laboratory make it clear that the lymphatic pathway is possible, although the lumen would appear to be the simpler path in most instances.

What determines the ultimate attack on the kidney? Perhaps the most important contributions from the experimental approach to pyelonephritis using the hematogenous system have been the uniform observations that the medulla is the most likely place in which bacterial multiplication first occurs The production of ammonia by the medulla provides one of the bases whereby the medulla becomes more susceptible to infection, and we may look forward to more detailed understanding of the enzymatic sequences accounting for this effect as well as to clearer views of the role of acidosis in susceptibility to infection. These observations of Beeson and Rowley, with further studies by Freedman, may offer critical information concerning the special susceptibility of the kidney to multiplication of gram-negative rods

The meticulous observations of Novikoff also indicate that acid-phosphatase-containing phagocytes are more abundant in the medulla than had generally been thought to be the case. The role of other factors, such as phenacetin and similar drugs, and of specifically heritable defects, as well as of other metabolic disturbances that may lead to diminution in renal resistance to bacterial multiplication, represents an important area for future investigation.

The rather specific involvement of the medulla, particularly if the infection ascends, accounts in a general way for the early loss of concentrating power that appears in pyelonephritis The careful observations of Bricker and his associates have made it likely that osmotic diuresis accounts for the loss of concentrating ability in more advanced renal disease. Their observations cannot account for findings such as those presented by Brod and Kaitz, in which loss of concentrating ability occurred in the absence of apparent loss of glomerular flow.

One of the major questions left unanswered is the relationship between acute and chronic active pyelonephritis on the one hand, and the scarred "burnt out" or healed or inactive chronic pyelonephritis on the other. The provocative questions raised by Kimmelstiel remain unanswered. It is difficult to view the present data without the feeling that not all inactive pyelonephritis began as active pyelonephritis. Since this problem also

in females over males, and obviously explains the induction of bacteriuria after instrumentation through the urethra. Bacteria may enter the urinary tract frequently via the urethra or may do so only under special circumstances. It is not possible at present to distinguish these two possibilities. If the former possibility is the correct one, it presupposes an antibacterial mechanism within the bladder. The argument for such a mechanism follows.

In general, pathogens of the urinary tract multiply well in urine, thus the introduction of bacteria into the bladder should be followed by geometric increases in bacterial numbers in the urine. Since the degree of dilution of bacteria as new urine enters the bladder is arithmetic, and a small volume of urine containing an inoculum of bacteria remains in the bladder after each micturition, it is difficult to see how bladders free themselves of bacteria without some inhibition of multiplication in the bladder. Any such inhibition would be expected to occur only when bacteria are adherent to mucosal cells at the end of micturition, and perhaps mucosal metabolic acids provide the postulated bacteriostatic effect. There can be no doubt, from a variety of clinical and experimental studies, that bladders can clear themselves of bacteria under circumstances that make simple dilution an inadequate explanation. If a local effect of bladder mucosa on bacterial multiplication were postulated, the effect of small amounts of residual urine on the bladder would consist of keeping bacteria from close contact with the mucosal cells. This would be sufficient to assure persistent bacteriuria once bacteria had gained entry to the bladder. Interference with the production of metabolic acid by drugs or by metabolic disturbances such as diabetes mellitus might provide another basis for the persistence of bacteriuria.

It would be comprehensible that trauma and disturbances in the vaginal bacterial flora might also influence the ease with which bacteria gained entry into the bladder.

Unanswered, however, is the problem of the predominance of the gram-negative rod in urinary tract infections when urethral organisms ordinarily are staphylococci, diphtheroids, enterococci, and so forth. It is difficult to accept such gross explanations as toilet habits to account for the predominance of coliform organisms in the urine in pyelonephritis. This rather mundane point may provide a key to the disease.

Once bacteria have begun to multiply in the bladder, it may be suggested that ascending pyelonephritis will occur in a certain number of instances. The experimental demonstration of ascension is complete, but only inferences concerning the human situation can be drawn at present. It is apparent from the observations of many workers – Gorrill, Guze, Braude, McCabe and Jackson – and from observations in our laboratory, that individual bacterial strains differ with respect to capacity to produce

manifest disease in the urinary tract. The implications of the studies of Edwards and of those cited by Rantz in his discussion are unmistakable. Detailed biologic study of strains of organisms obtained from patients with pyelonephritis is urgently needed, and at present we have no adequate markers for discerning biologic differences that relate to pathogenicity outside of the infected host.

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impinges on the problem of hypertension and on the host of metabolic disturbances accompanying renal failure, the need for clarification of this important area could hardly be greater.

The brilliant advances in understanding renal function that have occurred during the past few decades have only recently been related to pyelonephritis. Already it is clear that Wirz's hypothesis is the most satisfactory basis for explaining the selective loss of concentrating ability by the kidney in pyelonephritis. How the other functions of the kidney relate, and how further study of pyelonephritis will uncover abnormalities of function that may provide leads to understanding normal function of the kidney, remain to be seen. Certainly clinical investigators of the kidney have become increasingly interested in pyelonephritis simply because of the large numbers of cases that appear at every clinic devoted to the study of renal disease. In part, the diffuse nature of the bacterial attack on the kidney has led renal physiologists and microanatomists away from pyelonephritis and toward lesions such as nephrosis, in which the anatomic and physiologic disturbances are more sharply localized. If the present conference has stimulated correlative study in the more complex world of pyelonephritis, it will have served a most important function.

As is so often the case in scientific medicine, initial empiricisms and chance observations provide a basis for mechanistic attacks in new fields It can confidently be predicted that further progress in the study of pyelonephritis will bring about greater understanding of mechanisms of host resistance, more information concerning elements of microbial pathogenicity, greater insight into functional and corresponding structural changes in the kidney, better understanding of the role of the kidney in hypertension and in control of erythropoiesis and related functions – and, oddly enough, it may relate to such matters as mechanisms of prematurity, neonatal mortality, and hormone action. These broad byways have always resulted from sustained study of natural phenomena, and pyelonephritis has certainly proved to be no exception as a stimulus to observations at the broadest level of scientific exploration

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